Online ISSN : 2249-4626
Print ISSN : 0975-5896
DOI : 10.17406/GJSFR

Global Journal of Science Frontier Research: B

Chemistry

Highlights

- Hcl Binary System
- Methoxy Alkane Frankincense
- Sea Squirts and their Larvae
- Metal Complexes Synthesis

Discovering Thoughts, Inventing Future

VOLUME 16 ISSUE 2 VERSION 1.0

© 2001-2016 by Global Journal of Science Frontier Research, USA

Global Journal of Science Frontier Research: B
Chemistry

Global Journal of Science Frontier Research: B
Chemistry

Volume 16 Issue 2 (Ver. 1.0)

Open Association of Research Society

© Global Journal of Science Frontier Research. 2016.

All rights reserved.

This is a special issue published in version 1.0 of "Global Journal of Science Frontier Research." By Global Journals Inc.

All articles are open access articles distributed under "Global Journal of Science Frontier Research"

Reading License, which permits restricted use. Entire contents are copyright by of "Global Journal of Science Frontier Research" unless otherwise noted on specific articles.

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopy, recording, or any information storage and retrieval system, without written permission.

The opinions and statements made in this book are those of the authors concerned. Ultraculture has not verified and neither confirms nor denies any of the foregoing and no warranty or fitness is implied.

Engage with the contents herein at your own risk.

The use of this journal, and the terms and conditions for our providing information, is governed by our Disclaimer, Terms and Conditions and Privacy Policy given on our website http://globaljournals.us/terms-and-condition/menu-id-1463/

By referring / using / reading / any type of association / referencing this journal, this signifies and you acknowledge that you have read them and that you accept and will be bound by the terms thereof.

All information, journals, this journal, activities undertaken, materials, services and our website, terms and conditions, privacy policy, and this journal is subject to change anytime without any prior notice.

Incorporation No.: 0423089
License No.: 42125/022010/1186
Registration No.: 430374
Import-Export Code: 1109007027
Employer Identification Number (EIN):
USA Tax ID: 98-0673427

Global Journals Inc.

*(A Delaware USA Incorporation with "Good Standing"; **Reg. Number: 0423089**)*

Sponsors: Open Association of Research Society
Open Scientific Standards

Publisher's Headquarters office

Global Journals® Headquarters
945th Concord Streets,
Framingham Massachusetts Pin: 01701,
United States of America
USA Toll Free: +001-888-839-7392
USA Toll Free Fax: +001-888-839-7392

Offset Typesetting

Global Journals Incorporated
2nd, Lansdowne, Lansdowne Rd., Croydon-Surrey,
Pin: CR9 2ER, United Kingdom

Packaging & Continental Dispatching

Global Journals
E-3130 Sudama Nagar, Near Gopur Square,
Indore, M.P., Pin:452009, India

Find a correspondence nodal officer near you

To find nodal officer of your country, please email us at *local@globaljournals.org*

eContacts

Press Inquiries: *press@globaljournals.org*
Investor Inquiries: *investors@globaljournals.org*
Technical Support: *technology@globaljournals.org*
Media & Releases: *media@globaljournals.org*

Pricing (Including by Air Parcel Charges):

For Authors:
22 USD (B/W) & 50 USD (Color)
Yearly Subscription (Personal & Institutional):
200 USD (B/W) & 250 USD (Color)

Integrated Editorial Board
(Computer Science, Engineering, Medical, Management, Natural Science, Social Science)

John A. Hamilton,"Drew" Jr.,
Ph.D., Professor, Management
Computer Science and Software Engineering
Director, Information Assurance Laboratory
Auburn University

Dr. Henry Hexmoor
IEEE senior member since 2004
Ph.D. Computer Science, University at Buffalo
Department of Computer Science
Southern Illinois University at Carbondale

Dr. Osman Balci, Professor
Department of Computer Science
Virginia Tech, Virginia University
Ph.D.and M.S.Syracuse University, Syracuse, New York
M.S. and B.S. Bogazici University, Istanbul, Turkey

Yogita Bajpai
M.Sc. (Computer Science), FICCT
U.S.A.Email:
yogita@computerresearch.org

Dr. T. David A. Forbes
Associate Professor and Range Nutritionist
Ph.D. Edinburgh University - Animal Nutrition
M.S. Aberdeen University - Animal Nutrition
B.A. University of Dublin- Zoology

Dr. Wenying Feng
Professor, Department of Computing & Information Systems
Department of Mathematics
Trent University, Peterborough,
ON Canada K9J 7B8

Dr. Thomas Wischgoll
Computer Science and Engineering,
Wright State University, Dayton, Ohio
B.S., M.S., Ph.D.
(University of Kaiserslautern)

Dr. Abdurrahman Arslanyilmaz
Computer Science & Information Systems Department
Youngstown State University
Ph.D., Texas A&M University
University of Missouri, Columbia
Gazi University, Turkey

Dr. Xiaohong He
Professor of International Business
University of Quinnipiac
BS, Jilin Institute of Technology; MA, MS, PhD,. (University of Texas-Dallas)

Burcin Becerik-Gerber
University of Southern California
Ph.D. in Civil Engineering
DDes from Harvard University
M.S. from University of California, Berkeley & Istanbul University

Dr. Bart Lambrecht
Director of Research in Accounting and FinanceProfessor of Finance
Lancaster University Management School
BA (Antwerp); MPhil, MA, PhD (Cambridge)

Dr. Carlos García Pont
Associate Professor of Marketing
IESE Business School, University of Navarra
Doctor of Philosophy (Management), Massachusetts Institute of Technology (MIT)
Master in Business Administration, IESE, University of Navarra
Degree in Industrial Engineering, Universitat Politècnica de Catalunya

Dr. Fotini Labropulu
Mathematics - Luther College
University of ReginaPh.D., M.Sc. in Mathematics
B.A. (Honors) in Mathematics
University of Windso

Dr. Lynn Lim
Reader in Business and Marketing
Roehampton University, London
BCom, PGDip, MBA (Distinction), PhD, FHEA

Dr. Mihaly Mezei
ASSOCIATE PROFESSOR
Department of Structural and Chemical Biology, Mount Sinai School of Medical Center
Ph.D., Etvs Lornd University
Postdoctoral Training,
New York University

Dr. Söhnke M. Bartram
Department of Accounting and FinanceLancaster University Management SchoolPh.D. (WHU Koblenz)
MBA/BBA (University of Saarbrücken)

Dr. Miguel Angel Ariño
Professor of Decision Sciences
IESE Business School
Barcelona, Spain (Universidad de Navarra)
CEIBS (China Europe International Business School).
Beijing, Shanghai and Shenzhen
Ph.D. in Mathematics
University of Barcelona
BA in Mathematics (Licenciatura)
University of Barcelona

Philip G. Moscoso
Technology and Operations Management
IESE Business School, University of Navarra
Ph.D in Industrial Engineering and Management, ETH Zurich
M.Sc. in Chemical Engineering, ETH Zurich

Dr. Sanjay Dixit, M.D.
Director, EP Laboratories, Philadelphia VA Medical Center
Cardiovascular Medicine - Cardiac Arrhythmia
Univ of Penn School of Medicine

Dr. Han-Xiang Deng
MD., Ph.D
Associate Professor and Research Department Division of Neuromuscular Medicine
Davee Department of Neurology and Clinical NeuroscienceNorthwestern University Feinberg School of Medicine

Dr. Pina C. Sanelli
Associate Professor of Public Health
Weill Cornell Medical College
Associate Attending Radiologist
NewYork-Presbyterian Hospital
MRI, MRA, CT, and CTA
Neuroradiology and Diagnostic Radiology
M.D., State University of New York at Buffalo,School of Medicine and Biomedical Sciences

Dr. Roberto Sanchez
Associate Professor
Department of Structural and Chemical Biology
Mount Sinai School of Medicine
Ph.D., The Rockefeller University

Dr. Wen-Yih Sun
Professor of Earth and Atmospheric SciencesPurdue University Director National Center for Typhoon and Flooding Research, Taiwan University Chair Professor Department of Atmospheric Sciences, National Central University, Chung-Li, TaiwanUniversity Chair Professor Institute of Environmental Engineering, National Chiao Tung University, Hsin-chu, Taiwan.Ph.D., MS The University of Chicago, Geophysical Sciences
BS National Taiwan University, Atmospheric Sciences
Associate Professor of Radiology

Dr. Michael R. Rudnick
M.D., FACP
Associate Professor of Medicine
Chief, Renal Electrolyte and Hypertension Division (PMC)
Penn Medicine, University of Pennsylvania
Presbyterian Medical Center, Philadelphia
Nephrology and Internal Medicine
Certified by the American Board of Internal Medicine

Dr. Bassey Benjamin Esu
B.Sc. Marketing; MBA Marketing; Ph.D Marketing
Lecturer, Department of Marketing, University of Calabar
Tourism Consultant, Cross River State Tourism Development Department
Co-ordinator , Sustainable Tourism Initiative, Calabar, Nigeria

Dr. Aziz M. Barbar, Ph.D.
IEEE Senior Member
Chairperson, Department of Computer Science
AUST - American University of Science & Technology
Alfred Naccash Avenue – Ashrafieh

President Editor (HON.)

Dr. George Perry, (Neuroscientist)
Dean and Professor, College of Sciences
Denham Harman Research Award (American Aging Association)
ISI Highly Cited Researcher, Iberoamerican Molecular Biology Organization
AAAS Fellow, Correspondent Member of Spanish Royal Academy of Sciences
University of Texas at San Antonio
Postdoctoral Fellow (Department of Cell Biology)
Baylor College of Medicine
Houston, Texas, United States

Chief Author (HON.)

Dr. R.K. Dixit
M.Sc., Ph.D., FICCT
Chief Author, India
Email: authorind@computerresearch.org

Dean & Editor-in-Chief (HON.)

Vivek Dubey(HON.)
MS (Industrial Engineering),
MS (Mechanical Engineering)
University of Wisconsin, FICCT
Editor-in-Chief, USA
editorusa@computerresearch.org

Sangita Dixit
M.Sc., FICCT
Dean & Chancellor (Asia Pacific)
deanind@computerresearch.org

Suyash Dixit
(B.E., Computer Science Engineering), FICCTT
President, Web Administration and
Development , CEO at IOSRD
COO at GAOR & OSS

Er. Suyog Dixit
(M. Tech), BE (HONS. in CSE), FICCT
SAP Certified Consultant
CEO at IOSRD, GAOR & OSS
Technical Dean, Global Journals Inc. (US)
Website: www.suyogdixit.com
Email:suyog@suyogdixit.com

Pritesh Rajvaidya
(MS) Computer Science Department
California State University
BE (Computer Science), FICCT
Technical Dean, USA
Email: pritesh@computerresearch.org

Luis Galárraga
J!Research Project Leader
Saarbrücken, Germany

Contents of the Issue

i. Copyright Notice
ii. Editorial Board Members
iii. Chief Author and Dean
iv. Contents of the Issue

1. First Reporting on the Chemistry and Biological Activity of a Novel Boswellia Chemotype: The Methoxy Alkane Frankincense. *1-9*
2. Behavior Classification Physical/Chemistry Pa66/Hc*l* Binary System Utilized to Obtain Separation Membranes. *11-18*
3. Field and Laboratory Studies on Four Species of Sea Squirts and their Larvae. *19-45*

v. Fellows
vi. Auxiliary Memberships
vii. Process of Submission of Research Paper
viii. Preferred Author Guidelines
ix. Index

Global Journal of Science Frontier Research: B
Chemistry
Volume 16 Issue 2 Version 1.0 Year 2016
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

First Reporting on the Chemistry and Biological Activity of a Novel Boswellia chemotype: The Methoxy Alkane Frankincense

By Prabodh Satyal & Robert S. Pappas
Essential Oil University LLC, United States

Abstract- Two oleogum resin essential oils (from two different seasons: fall and summer), of *Boswellia spp.* (collected from Somalia), were obtained by hydrodistillation and analyzed by GC-MS. Out of 147 peaks, components were identified among the two essential oils accounting for 100%, and 99.7% of the oils, respectively. The two essential oils were dominated by the 1-methoxy alkane [octyl methyl ether (5.5-11.7%), and decyl methyl ether (30.6-54.9%)], α-pinene (0.3-11.5%), sabinene (2.1-7.2%), and α-bourbonene(1.7-5.7%). This is the first report of a methoxy alkane chemotyped frankincense essential oils as well as the first reporting of the natural occurance of decyl methyl ether (methyoxy decane) and octyl methyl ether (methyoxy octane). Monoterpenes chiral distributions were also measured and it was found that both of the oils have same enantiomeric ratio. Large chemical variation was attributed to seasonal variation. The essential oil harvested on fall season had also exhibited notable antimicrobial activities [*Aspergillus niger* (MIC = 39 μg/mL), *Candida albicans* (MIC = 78 μg/mL), *Bacillus cereus* (MIC = 78 μg/mL), *Staphylococus aureus* (MIC = 78 μg/mL), and *Escherichia coli* (MIC = 78 μg/mL)], the essential oil also showed pronounced cytotoxic activities (100% kill on MCF-7 cells at 100 μg/mL).

Keywords: decyl methyl ether, frankincense, cytotoxicity, antimicrobial, enantiomeric distribution, methoxy alkane.

GJSFR-B Classification : FOR Code: 250499

FIRSTREPORTINGONTHECHEMISTRYANDBIOLOGICALACTIVITYOFANOVELBOSWELLIACHEMOTYPETHEMETHOXYALKANEFRANKINCENSE

Strictly as per the compliance and regulations of :

© 2016. Prabodh Satyal & Robert S. Pappas. This is a research/review paper, distributed under the terms of the Creative Commons Attribution-Noncommercial 3.0 Unported License http://creativecommons.org/licenses/by-nc/3.0/), permitting all non commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

First Reporting on the Chemistry and Biological Activity of a Novel Boswellia chemotype: The Methoxy Alkane Frankincense

Prabodh Satyal [α] & Robert S. Pappas [σ]

Abstract- Two oleogum resin essential oils (from two different seasons: fall and summer), of *Boswellia spp.* (collected from Somalia), were obtained by hydrodistillation and analyzed by GC-MS. Out of 147 peaks, components were identified among the two essential oils accounting for 100%, and 99.7% of the oils, respectively. The two essential oils were dominated by the 1-methoxy alkane [octyl methyl ether (5.5-11.7%), and decyl methyl ether (30.6-54.9%)], α-pinene (0.3-11.5%), sabinene (2.1-7.2%), and α-bourbonene (1.7-5.7%). This is the first report of a methoxy alkane chemotyped frankincense essential oils as well as the first reporting of the natural occurance of decyl methyl ether (methyoxy decane) and octyl methyl ether (methyoxy octane). Monoterpenes chiral distributions were also measured and it was found that both of the oils have same enantiomeric ratio. Large chemical variation was attributed to seasonal variation. The essential oil harvested on fall season had also exhibited notable antimicrobial activities [*Aspergillus niger* (MIC = 39 µg/mL), *Candida albicans* (MIC = 78 µg/mL), *Bacillus cereus* (MIC = 78 µg/mL), *Staphylococus aureus* (MIC = 78 µg/mL), and *Escherichia coli* (MIC = 78 µg/mL)], the essential oil also showed pronounced cytotoxic activities (100% kill on MCF-7 cells at 100 µg/mL).

Keywords: decyl methyl ether, frankincense, cytotoxicity, antimicrobial, enantiomeric distribution, methoxy alkane.

I. Introduction

Boswellia spp (also known as frankincense or Olibanum) is one of the most popular essential oils in aromatherapy from the Bursearaceae family. word frankincense is derived from an ancient French word which means "Franc = pure + enens=incense" and is mentioned frequently in many sacred text including the Bible. Frankincense essential oil is obtained from a variety different species of the genus *Boswellia.* The most prevalent species in today's world market are *B. carterii (Somaliland), B. serrata (India), B. sacra (Oman), B. frereana (Somaliland)* and *B. papyrifera (Ethiopia).* The bark of the *Boswellia* tree is filled with oleo-gum-resin reservoirs. When the reservoirs are penetrated, a milky juice is excreted onto the bark. When the milky juice becomes exposed to air it begins to harden producing the oleogum resin. [Tucker, 1986]. The resin typically contains between 5 and 15% essential oil [Mertens, et al., 2009]. Frankincense oil is used medicinally in many cultures still today. For example, frankincense essential oil has been claimed to contain chemical components present that aid in the removal of scars and stretch marks, in addition to having antibacterial and antifungal properties [Mikhaeil, et al.,2003]. The oil has also been studied in reference to pharmaceutical properties as well as clinical trials [Vuuren, et al., 2010].

Frankincense resin is traditionally used in treatment of inflammation, wound healing, skin diseases, urinary tract infections, etc. Its application in medicines and cosmetics product formulation are increasing daily.

For a number of years there has been considerable controversy [Paul, et al., 2011, Woolley, et al., 2012] in identifying the correct botanical name of certain frankincense species. Paul et al have proposed a simple TLC method for identification of three olibanum resins on the basis of biomarker compounds. According to their findings, the presence of incensole and incensyl acetate confirms the presence of *Boswellia papyrifera*, caryophyllene oxide confirms *Boswellia carterii* and/or *Boswellia sacra*, *Boswellia serrata* has neither incensole acetate nor caryophyllene oxide, but has a remarkable amount of serratol present in addition to trace amount of incensole. It has been claimed for some time that *B. sacra* is actually the same as *B. carterii* [Thulin and Warfa, 1987], but the enantiomeric studies by Woolley et al [Wooley, et al., 2012] as well as the finding of this laboratory give strong evidence that they are indeed different species.

The current study was conducted to report the unique chemical composition (first time presence of alkyl methyl ether as the natural volatile component in an essential oil as well as first reporting of its presence in nature in general), enantiomeric distribution, and biological activities of the oleogum resin essential oil of *Boswellia spp* from Somalia.

Author α : Essential Oil University, New Albany IN 47150, USA.
e-mail: dr.p@essentialoils.org

Global Journal of Science Frontier Research (B) Volume XVI Issue II Version I Year 2016

© 2016 Global Journals Inc. (US)

Picture 1 : Methoxy alkane chemotyped *Boswellia* resin in collected in Northern Somaliland

II. Essential Oils Composition

The Frankincense oil was obtained in 1-1.5% yields, a notably lower oil yield than the typical resin from Somaliland. A total of 147 compounds were identified, accounting for about 100% of compound identification (see Table 1). The essential oils contained α-pinene (0.3-11.6%), sabinene (2.1-7.2%), octyl methyl ether (5.5-11.7%), decyl methyl ether (30.6-54.9%), and α-bourbonene (1-5.7%). These results are qualitatively different than any result previously published in a significant review paper [Mertens, et al., 2009; Niebler & Buttner, 2016; Niebler et al., 2016]. On analyzing twenty commercial essential oil samples (mostly carterii, neglecta, sacra, thurifera, frereana) from South Africa, revealed those oils were composed of α-pinene (2.0-64.7%), α-thujene (0.3-52.4%), β-pinene (0.3-13.1%), myrcene (1.1-22.4%), sabinene (0.5-7.0%), limonene (1.3-20.4%), p-cymene (2.7-16.9%), and β-caryophyllene (0.1-10.5%) [Vuuren et al. 2010]. None of the samples exhibited the presence of methoxy alkane (octyl methyl ether, decyl methyl ether) even in trace quantities. Frankincense the North east region of Somalia has also been studied and according to this report α-pinene (10.3-37.7%), α-phellandrene (12.2-41.8%), limonene (6.4-19.6%) were the major components [Vuuren, et al., 2010]. Presence of 9.84% decyl methyl ether (Table-4) in dichloromethane extract indicated natural occurrence of decyl methyl ether in *Boswellia* spp.

It has been shown that enantiomeric distribution is important in properly identifying frankincense species [Woolley et al., 2012], so on our enantiomeric studies of two resin samples harvested at different times show similar enantiomeric distribution from each other as shown in table 3.

In addition to genetic variation, other factors such as age, vegetative cycle stage, climate, season, soil composition, etc. are among several things responsible for the considerable variation in essential oil compositions [Satyal, et al., 2012]. Based on the observed chemical composition, this variety of Somaliland frankincense may be treated as a distinct and novel chemotype.

III. Cytotoxicity, Antimicrobial Activity

Frankincense oil showed notable biological activity on all of the tested microorganisms (Table 3): *Bacillus cereus* (MIC = 78 μg/mL), *Staphylococcus aureus* (MIC = 78 μg/mL), *Escherichia coli* (MIC = 78 μg/mL) and *Aspergillus niger* (MIC = 39 μg/mL). Several previous studies have reported antibacterial and antifungal [Vuuren et al 2010] activities for *Frankincense* essential oils, consistent with our results. α–pinene and

© 2016 Global Journals Inc. (US)

decyl methyl ether, especially in synergy with other essential oil components, are likely responsible for the antimicrobial activities as shown in the table 3.

Frankincense essential oil demonstrated notable *in-vitro* cytotoxic activity against MCF-7 breast tumor cells (100% kill at 100 µg/mL). Note that decyl methyl ether, α-pinene, and β-pinene, are not appreciably cytotoxic, either alone (Table 3).

Table 1 : Chemical Composition of methoxy alkane chemotyped Frankincense EO from Somaliland

RI[1]	Compounds	%[2a] Fall	%[2b] Summer
821	Hexyl methyl ether	TR	ND
863	n-Hexanol	TR	ND
881	2-Butyl furan	TR	ND
900	Nonane	TR	ND
919	Hasheshene	0.10	ND
921	Tricyclene	0.03	ND
924	α-Thujene	2.01	0.38
932	α-Pinene	11.55	0.34
941	Thujadiene	0.05	ND
948	Camphene	0.37	ND
951	Thuja-2,4(10)-diene	0.21	ND
953	α-Fenchene	0.02	ND
971	Sabinene	7.20	2.1
976	β-Pinene	0.56	0.04
982	Hept-5-en-2-one <6-methyl->	0.02	ND
988	Myrcene	0.26	0.04
1003	Octanal	ND	0.02
1004	Pseudolimonene	0.10	ND
1006	α-Phellandrene	0.87	0.06
1008	3-Carene	0.19	ND
1011	Hexyl acetate	ND	0.01
1014	1,4-Cineole	0.02	0.01
1016	α-Terpinene	0.49	0.17
1023	*p*-Cymene	1.58	0.61
1025	Octyl methyl ether	5.53	11.72
1028	Limonene	1.45	0.13
1030	β-Phellandrene	1.16	ND
1031	1,8-cineole	0.11	ND
1034	(*Z*)-β-Ocimene	0.13	0.04
1036	2,5-dimethylcyclohexanol A	0.05	0.03
1042	2,5-Dimethylcyclohexanol B	ND	0.06
1045	(*E*)-β-Ocimene	0.13	ND
1057	γ-Terpinene	0.88	0.31
1066	*o*-Cymenene	TR	0.04
1069	Octanol	0.09	0.39
1075	2-Decanol methyl ether	0.21	0.41
1080	Unidentified	ND	0.07
1084	Terpinolene	0.19	0.05
1089	*p*-Cymenene	0.10	0.03

© 2016 Global Journals Inc. (US)

RI[1]	Compounds	%[2a] Fall	%[2b] Summer
1099	Linalool	0.19	0.09
1101	2-Nonanol	TR	0.04
1112	4,8 Dimetyl-nona-1,3,7-triene	0.13	0.46
1117	β-Thujone	TR	ND
1118	3-Octyl acetate	TR	0.01
1121	*cis*-Non-3-en-1-ol, methyl ether	TR	ND
1123	*cis-p*-Menth-2-en-1-ol	0.06	ND
1126	Methyl nonyl ether	1.37	2.21
1128	Hexyl propanoate	0.11	0.07
1138	*trans*-Sabinol	TR	ND
1139	*trans*-Pinocarveol	0.22	ND
1140	*cis*-Verbenol	0.12	ND
1142	Camphene hydrate	ND	0.02
1144	*trans*-Verbenol	0.27	ND
1149	α-Felandren-8-ol	0.13	ND
1156	Sabina ketone	TR	ND
1158	β-Pinene oxide	TR	ND
1159	*trans*-Pinocamphone	TR	ND
1168	α-Phellandrene epoxide	TR	ND
1170	*p*-Mentha-1,5-dien-8-ol	0.32	ND
1171	n-Nonanol	ND	0.05
1179	Terpinen-4-ol	1.27	0.47
1185	*p*-Cymen-8-ol	0.07	0.02
1191	Hexyl butanoate	0.17	0.14
1194	α-Terpineol	0.24	0.02
1206	*cis*-Decen-9-ol methyl ether	0.34	0.34
1207	*trans*-Piperitol	TR	ND
1208	(2*E*,4*E*)-Methyl dodeca-2,4-dienoate	ND	0.03
1209	Octyl acetate	0.15	0.58
1214	*trans*-Decen-9-ol, methyl ether	0.26	0.4
1218	*cis*-Decen-1-ol, methyl ether	0.37	0.74
1222	trans-Decen-1-ol, methyl ether	ND	0.06
1227	Decyl methyl ether	30.65	54.91
1242	*cis*-Dec-2-en-1-ol, methyl ether	0.72	1.17
1248	Linalyl acetate	TR	ND
1252	Piperitone	ND	0.02
1262	*trans*-Dec-2-enal	ND	0.02
1271	Decanol	0.63	2.1
1283	Bornyl acetate	0.27	ND
1319	*cis*-Undecen-1-ol, methyl ether	0.11	0.09
1322	Methyl decanoate	0.00	0.03
1326	Undecyl methyl ether	0.20	0.29
1345	α-Cubebene	0.59	0.32
1367	α-Ylangene	0.37	0.28
1374	α-Copaene	1.68	1.74

© 2016 Global Journals Inc. (US)

RI[1]	Compounds	%[2a] Fall	%[2b] Summer
1382	α-Bourbonene	5.72	1.69
1385	Hexyl hexanoate	0.89	0.21
1387	β-Elemene	1.12	0.24
1390	Sativene	0.07	0.05
1393	1,5-di-epi-α-Bourbonene	0.13	ND
1402	*cis*-Caryophyllene	0.07	0.02
1405	α-Gurjunene	0.20	0.11
1408	Decyl acetate	0.09	1.18
1416	β-Ylangene	0.43	0.12
1417	*trans*-Caryophyllene	0.83	0.72
1428	β-Copaene	0.75	0.22
1442	α-Aromadendrene	0.29	0.07
1447	*cis-Muurola-3,5-diene*	0.13	0.13
1453	α-Humulene	0.32	0.18
1458	Alloaromadendrene	0.20	0.17
1466	β-Aromadendrene	0.15	0.08
1470	*cis*-Cadina-1(6),4-diene	0.11	0.07
1473	*trans*-Cadina-1(6),4-diene	0.73	0.64
1476	*cis*-4,10-epoxy-Amorphane	TR	0.03
1479	Germacrene D	1.52	1.23
1483	Heptylhexanoate	ND	0.02
1487	β-Selinene	0.48	0.07
1489	*trans*-Muurola-4(14),5-diene	0.24	0.19
1493	α-Selinene	0.41	0.23
1496	α-Muurolene	0.31	0.21
1500	γ-Amorphene	0.06	0.03
1503	β-Dihydroagarofuran	0.04	0.03
1511	δ-Amorphene	0.20	0.16
1513	Cubebol	0.30	0.45
1516	δ-Cadinene	1.09	0.82
1520	Tridecyl methyl ether	0.38	0.5
1522	Methyl dodecanoate	TR	ND
1527	Kessane	0.06	0.07
1530	*trans*-Cadine-1,4-diene	0.06	0.05
1534	Isokessane	0.07	0.07
1539	Italicene ether	ND	0.02
1539	α-Calcorene	0.02	0.02
1546	α-Elemol	0.20	0.22
1557	Dodecanoic acid	ND	0.06
1559	*cis*-Muurol-5-en-4-α-ol+*trans*-Nerolidol	0.69	0.88
1565	Zierone	ND	0.02
1568	Caryophyllenol	0.05	0.11
1570	*trans*-Tridec-2-en-1-al	0.06	0.11
1576	Germacrene-D-4-ol	ND	0.02
1579	Caryophyllene oxide	TR	ND

© 2016 Global Journals Inc. (US)

RI[1]	Compounds	%[2a] Fall	%[2b] Summer
1592	Viridiflorol	0.11	ND
1602	Ledol	TR	0.03
1606	1,10 di-*epi*-Cubenol	0.24	0.16
1611	8-*epi*-γ-Eudesmol	1.10	1.38
1625	1-*epi*-Cubenol	TR	0.03
1641	Cubenol	0.11	0.03
1641	epi-α-Cadinol	ND	0.01
1644	δ-Cadinol	TR	0.03
1652	Unidentified Sesquiterpineol	ND	0.26
1653	α-Eudesmol	0.20	0.15
1673	Bulnesol	TR	0.06
1696	Tridec-4-en-1-yl acetate	ND	0.02
1791	α-Phellandrene dimer	0.05	ND
1950	*3E*-Cembrene A	0.49	0.31
1995	α-Pinacene	0.09	0.06
2007	Verticilla-4(20),7,11-triene	0.33	0.14
2131	Neocembrene A	0.14	0.12
2142	Incensole	0.79	0.43
2144	Serratol	0.41	0.55
	Total identified	100%	99.74%

Note: Where RI[1] Retention Index determined to a series of n-Alkanes on DB-5 column; compounds are listed in order of elution (Increasing RI), %[2a] and %[2b] refers to Percent of total oil collected in fall season and percent of total oil collected in summer season respectively. "TR" indicates trace components (<0.01%), and "ND" indicates non detected compounds on the provided conditions.

Table 2 : Enantiomeric Distribution of Frankincense EO collected in fall and summer season from Somalia

Monoterepene Components	Fall	Summer
(1R)+-α-thujene: (1S)-(-)-α-thujene	43 to 57	36 to 64
(1R,5R)-(+)-α-pinene: (1S,5S)-(–)-α-pinene	83 to 17	76 to 34
(4R)-(+)-limonene :(4S)-(-)-limonene	73 to 27	65 to 35
(4S-(+)-terpinen-4-ol : (4R)-(-)-terpinen-4-ol	81 to 19	71 to 29
(4S)-(+)-sabinene :(4R)-(-)-sabinene	93 to 7	98 to 2

Table 3 : Biological activities of *Frankincense* essential oils and major essential oil components

Bioassay	EO	Methoxydecane	α-Pinene	β-Pinene
MCF-7 cytotoxicity (% kill at 100 μg/mL)	100±1.6	80±10.6	16.8±2.6	30.4±7.7
Antimicrobial (MIC, μg/mL)				
Bacillus cereus	78	78	1250	1250
Staphylococcus aureus	78	313	1250	1250
Escherichia coli	78	78	1250	1250
Aspergillus niger	39	313	156	156
Candida albicans	78	313	625	313

© 2016 Global Journals Inc. (US)

Table 4 : Chemical composition of dichlromethane extract of methoxy alkane chemotyped frankincense resin from Somalia

RI[a]	Compounds	%[b]
924	α-Thujene	0.26
932	α-Pinene	0.26
971	Sabinene	1.08
1024	*p*-Cymene	0.13
1025	Octyl methyl ether	3.34
1057	γ-Terpinene	0.06
1075	2-Decanol, methyl ether	0.09
1112	2-Methyl-6-methylen-octa-1,7-dien-3-one	0.11
1126	Methyl nonyl ether	0.39
1209	Octyl acetate	0.06
1214	*cis*-Decen-1-ol, methyl ether	0.07
1218	*trans*-Decen-1-ol, methyl ether	0.11
1227	Decyl methyl ether	9.84
1242	*cis*-Dec-2-en-1-ol, methyl ether	0.21
1271	Decanol	0.47
1375	α-Copaene	0.36
1383	α-Bourbonene	0.35
1408	Decyl acetate	0.24
1419	*trans*-Caryophyllene	0.11
1474	*trans*-Cadina-1(6),4-diene	0.18
1480	Germacrene D	0.37
1494	*epi*-Cubebol	0.07
1514	Cubebol	0.47
1517	δ-Cadinene	0.2
1520	Tridecyl methyl ether	0.17
1547	Elemol	0.32
1558	Dodecanoic acid	0.41
1561	Prenopsan-8-ol	0.75
1570	*2E*-Tridecen-1-al	0.16
1608	1,10-di-*epi*-Cubenol	0.18
1613	5-*epi*-7-*epi*-α-Eudesmol	2.48
1652	8-epi-γ-Eudesmol	0.67
1654	α-Eudesmol	0.36
1674	iso-Bulensol	0.1
1952	*3E*-Cembrene A	1.36
1996	α-Pinacene	0.43
2009	Verticilliol	0.72
2133	Neocembrene A	7.1
2145	Incensole	43.39
2147	Serratol	20.23
2253	Incensole oxide A	0.2
2263	Incensole oxide B	1.04
2269	Isoincensole	0.46
2293	Isoincensole oxide	0.35
2350	Cembra-2,7,11-trien-4,5-diol isomer	0.29
	Total Identified	100%

© 2016 Global Journals Inc. (US)

IV. Experimental

a) Plant Material

Oleo-gum-resin were collected from the city Ufeyn (10.6500° N, 49.7500° E, 470 m above sea level) in the Puntland region of Somalia for two times of year [2014 April 20th and 2015 September 9th].

The voucher specimen of resin collected plant was stored in, Somalia. Local botanist has identified this resin as *Boswellia carterii,* but on the basis of previously published articles, it did not match chemical composition, so throughout of this article, we mention it as Frankincense or Boswellia spp to avoid controversy in taxonomy. The air-dried sample (250 g) were hydrodistilled using a Clevenger Apparatus for 4 hours to yield a translucent, yellow essential and colorless oil. The essential oil was stored at room temperature until analysis was carried out.

In addition to steam distillation, this resin was also extracted with methylene chloride and analyzed by GC/MS (see Table-4) in order to confirm the presence of the methoxy alkane components in the resin itself. This experiment was done to avoid any possible arguments against these components occurring naturally, ruling out the possibility that the methoxy alkanes are arising from a secondary reaction occurring during the distillation process.

b) Gas Chromatographic-Mass Spectral Analysis

The essential oil of Boswellia was analyzed by GC-MS using a Shimadzu GCMS-QP2010 Ultra operated in the EI mode [(electron energy = 70eV), scan range = 3.0 scans/sec], and GCMS Solution software. The GC column was Zebron ZB-5MS fused silica capillary column with a (5% phenyl)-polymethyl siloxane stationary phase a film thickness of 0.25 mm. The career gas was helium with a column head pressure 80 psi and flow rate of 1.37 ml/min. Injector temperature was 250°C and the ion source temperature was 200°C, increase in temperature rate 2°C/min to 260°C. The GC oven temperature program was programmed for 50°C initial temperature, increase in rate 2°C/min to 260°C. A 5% w/v solution of the sample in CH_2Cl_2 was prepared and 0.1 μL was injected in splitting mode (30:1). Identification of the oil components was based on their retention indices determined by reference to a homologous series of n-alkanes, and by comparison of their mass spectral fragmentation patterns with those reported in the literature [Adams], and stored in the MS library.

c) Chiral Gas Chromatographic-Mass Spectral Analysis

Chiral analysis of the essential oils was performed on a Shimadzu GCMS-QP2010S operated in the EI mode [(electron energy=70eV), scan range = 3.0 scans/sec]. GC equipped with a RestekB-Dex 325 capillary column (30 m×0.25 mm ID×0.25 _m film). Oven temperature was started at 50◦C, and then gradually raised to 120◦C at 1.5 ◦C/min. The oven was then raised to 200◦C at 2◦C/min and held for 5 min. Helium was the carrier gas and flow rate was maintained at 1.8 ml/min. Samples were diluted 3% w/v with CH_2Cl_2 and then a 0.1 μL sample was injected in a split mode with a split ratio of 1:45.

d) Antimicrobial Screening

The essential oil was screened for antimicrobial activity against Gram-positive bacteria, *Bacillus cereus* (ATCC No. 14579) and *Staphylococcus aureus* (ATCC No. 29213); Gram-negative bacteria, *Pseudomonas aeruginosa* (ATCC No. 27853) and *Escherichia coli* (ATCC No. 10798). Minimum inhibitory concentrations (MICs) were determined using the microbroth dilution technique [Satyal et al., 2013]. Dilutions of the crude extracts were prepared in cation-adjusted Mueller Hinton broth (CAMHB) beginning with 50 μL of 1% w/w solutions of crude extracts in DMSO plus 50 μL CAMHB. The extract solutions were serially diluted (1:1) in CAMHB in 96-well plates. Organisms at a concentration of approximately 1.5×10^8 colony-forming units (CFU)/mL were added to each well. Plates were incubated at 37°C for 24 hours; the final minimum inhibitory concentration (MIC) was determined as the lowest concentration without turbidity. Geneticin® was used as a positive antibiotic control; DMSO was used as a negative control. Antifungal activity against *Aspergillus niger* (ATCC No. 16888) was determined as above using YM broth inoculated with *A. niger* hyphal culture diluted to a McFarland turbidity of 1.0. Amphotericin B was the positive control.

e) Cytotoxic Activity

Human MCF-7 breast adenocar-cinoma cells (ATCC No. HTB-22) [Satyal et al, 2014] were grown in a 3% CO_2 environment at 37°C in RPMI-1640 medium, supplemented with 10% fetal bovine serum, 100,000 units penicillin and 10.0 mg streptomycin per liter of medium, 15mM of Hepes, and buffered with 26.7 mM $NaHCO_3$, pH 7.35. Cells were plated into 96-well cell culture plates at 2.5×10^4 cells per well. The volume in each well was 100 µL. After 48 h, supernatant fluid was removed by suction and replaced with 100 µL growth medium containing 1.0 µL of DMSO solution of the essential oil (1% w/w in DMSO), giving a final concentration of 100 µg/mL for each well. Solutions were added to wells in four replicates. Medium controls and DMSO controls (10 µL DMSO/mL) were used. Tingenone [Satyal et al, 2015] was used as a positive control. After the addition of compounds, plates were incubated for 48 h at 37°C in 5% CO_2; medium was then removed by suction, and 100 µL of fresh medium was added to each well. In order to establish percent kill rates, the MTT assay for cell viability was carried out [Satyal et al., 2012]. After colorimetric readings were recorded (using a Molecular Devices SpectraMAX Plus

© 2016 Global Journals Inc. (US)

microplate reader, 570 nm), average absorbances, standard deviations, and percent kill ratios ($\%kill_{cmpd}/\%kill_{DMSO}$) were calculated.

V. Acknowledgments

PS is grateful to Bhuwan Chhetri, Dr. Noura S Dosoky, and Barkhad Hassan for their valuable help during biological screening and resin collection. RSP and PS are thankful to Tanner Wortham for his valuable help during distillation.

References Références Referencias

1. Adams, R. P. (2007). *Identification of Essential Oil Components by Gas Chromatography*. Illinois: Allured.
2. Groenendijk, P., Eshete, A., Sterck, F. J., Zuidema, P. A., & Bongers, F. (2011). Limitations to sustainable frankincense production: Blocked regeneration, high adult mortality and declining populations. *Journal of Applied Ecology,49*(1), 164-173.
3. Mertens, M., Buettner, A., & Kirchhoff, E. (2009). The volatile constituents of frankincense - a review. *Flavour and Fragrance Journal,24*(6), 279-300.
4. Mikhaeil, B. R., Maatooq, G. T., Badria, F. A., & Amer, M. M. (2003). Chemistry and Immuno-modulatory Activity of Frankincense Oil. *Zeitschrift Für Naturforschung C,58*(3-4).
5. Niebler, J., & Buettner, A. (2016). Frankincense Revisited, Part I: Comparative Analysis of Volatiles in Commercially Relevant Boswellia Species. *Chemistry & Biodiversity.*
6. Niebler, J., Eslamieh, J., & Buettner, A. (2016). Frankincense Revisited, Part II: Volatiles in Rare Boswellia Species and Hybrids. *Chemistry & Biodiversity*.
7. Paul, M., Brüning, G., Bergmann, J., & Jauch, J. (2011). A Thin-layer Chromatography Method for the Identification of Three Different Olibanum Resins (Boswellia serrata, Boswellia papyrifera and Boswellia carterii, respectively, Boswellia sacra). *Phytochemical Analysis,23*(2), 184-189.
8. Satyal, P., Paudel, P., Limbu, K., & Setzer, W. N. (2012). Leaf Essential Oil Composition of Citrus japonica from Nepal. *Journal of Essential Oil Bearing Plants,15*(3), 357-359.
9. Satyal, P., Satyal, P., Paudel, P., Raut, J., Deo, A., Dosoky, N. S., & Setzer, W. N. (2013). Volatile constituents of Pinus roxburghii from Nepal. *Pharmacognosy Research,5*(1), 43.
10. Satyal, P., & Setzer, W. N. (2014). Chemotyping and Determination of Antimicrobial, Insecticidal, and Cytotoxic Properties of Wild-Grown Cannabis sativa from Nepal. *Journal of Medicinally Active Plants,3*(1), 9-16.
11. Satyal, P., Chhetri, B. K., Dosoky, N. S., Poudel, A., & Setzer, W. N. (2015). Chemical Composition of Nardostachys grandiflora Rhizome Oil from Nepal-A Contribution to the Chemotaxonomy and Bioactivity of Nardostachys. *Natural Product Communications, 10*(6), 1067-1070.
12. Thulin, M., & Warfa, A. M. (1987). The Frankincense Trees (Boswellia spp., Burseraceae) of Northern Somalia and Southern Arabia. *Kew Bulletin,42*(3), 487.
13. Tucker, A. O. (1986). Frankincense and myrrh. *Economic Botany,40*(4), 425-433.
14. Vuuren, S. V., Kamatou, G., & Viljoen, A. (2010). Volatile composition and antimicrobial activity of twenty commercial frankincense essential oil samples. *South African Journal of Botany,76*(4), 686-691.
15. Woolley, C. L., Suhail, M. M., Smith, B. L., Boren, K. E., Taylor, L. C., Schreuder, M. F., . . . Young, D. G. (2012). Chemical differentiation of *Boswellia sacra* and Boswellia carterii essential oils by gas chromatography and chiral gas chromatography–mass spectrometry. *Journal of Chromatography A,1261*, 158-163.

© 2016 Global Journals Inc. (US)

This page is intentionally left blank

© 2016 Global Journals Inc. (US)

Global Journal of Science Frontier Research: B
Chemistry
Volume 16 Issue 2 Version 1.0 Year 2016
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Behavior Classification Physical/Chemistry PA66/HCℓ Binary System Utilized to Obtain Separation Membranes

By Jocelei Duarte, Venina dos Santos & Mara Zeni
Caxias do Sul University

Abstract- The dissolution of polymers in solvents is an interest area of polymers science and technology due to their applications in films and membranes. The starting point for the preparation of polymer membranes by phase inversion is having a thermodynamically stable solution. In this work, the thermodynamic behavior of polyamide 66 (PA66) solutions in formic acid (FA) and in hydrochloric acid (HCℓ), was studied to evaluate its physical/chemistry classification. The polymer systems may be divided into five classes (types), which incorporate polymer-low molecular liquid systems, ranging from true solutions to dispersions of polymers in liquids. The polyamide 66/formic acid (PA66/FA) binary system is considered a true solution. Several experiments were performed to verify if the polyamide 66/hydrochloric acid (PA66/HCℓ) binary system has all characteristics of true solution. The analysis showed that the PA66/HCℓ binary system presents all characteristics of true solution, including the final morphology of the membranes that are similar obtained membranes by PA66/FA binary system.

Keywords: polymer-low molecular liquid systems, true solution, PA66/HCℓ binary system.

GJSFR-B Classification : FOR Code: 250299p

BEHAVIORCLASSIFICATIONPHYSICALCHEMISTRYPA66HCLBINARYSYSTEMUTILIZEDTOOBTAINSEPARATIONMEMBRANES

Strictly as per the compliance and regulations of :

© 2016. Jocelei Duarte, Venina dos Santos & Mara Zeni. This is a research/review paper, distributed under the terms of the Creative Commons Attribution-Noncommercial 3.0 Unported License http://creativecommons.org/licenses/by-nc/3.0/), permitting all non commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Behavior Classification Physical/Chemistry PA66/HCℓ Binary System Utilized to Obtain Separation Membranes

Jocelei Duarte [a], Venina dos Santos [a] & Mara Zeni [a*]

Highlights

- The thermodynamic equilibrium is reached ($\Delta\mu = 0$ or $= \mu_1$) the solution becomes homogeneous.
- The PA66/HCℓ solution was frozen and thawedand the solution remained homogeneous.
- The Gibbs free energy of PA66/HCℓ system indicates to be more favorable to PA66/FA system.
- In PA66/HCℓ system dipole-dipole interactions occur, justifying the behavior similar to polyelectrolyte.
- The PA66 solution in HCℓ behaves as a true solution.

Abstract- The dissolution of polymers in solvents is an interest area of polymers science and technology due to their applications in films and membranes. The starting point for the preparation of polymer membranes by phase inversion is having a thermodynamically stable solution. In this work, the thermodynamic behavior of polyamide 66 (PA66) solutions in formic acid (FA) and in hydrochloric acid (HCℓ), was studied to evaluate its physical/chemistry classification. The polymer systems may be divided into five classes (types), which incorporate polymer-low molecular liquid systems, ranging from true solutions to dispersions of polymers in liquids. The polyamide 66/formic acid (PA66/FA) binary system is considered a true solution. Several experiments were performed to verify if the polyamide 66/hydrochloric acid (PA66/HCℓ) binary system has all characteristics of true solution. The analysis showed that the PA66/HCℓ binary system presents all characteristics of true solution, including the final morphology of the membranes that are similar obtained membranes by PA66/FA binary system.

Keywords: polymer-low molecular liquid systems, true solution, PA66/HCℓ binary system.

I. Introduction

The population world growth, as well as their level of requirement, both in quality and quantity, led the industry to produce ever more food, medicament, clothing and all kinds of durable goods and nondurable needed, directly and indirectly, to man. Facing this reality, new technologies have emerged to meet this demand and requirements of the population, in this context, Membrane Separation Processes (MSP) emerged as an alternative based on clean technologies, with its modular features and low energy.

The main advantage of the MSP is the separation occurs without phase change of components, different from the classical separation processes such as distillation. So, besides being economically more advantageous in a energy lower cost, the MSP can be used in very common in areas biomedical separation of thermolabile substances in juice concentration, where the use of thermal processes alter the organoleptic properties, among others[1]. Another advantage of this process is simplicity of operation, compact equipment, easy to mix with other processes and an increase in the production scale [2].

The boundary for industrial and commercial growth of MSP was the development of asymmetric membranes of cellulose acetate by researchers Loeb and Sourirajan (1961), using the phase inversion technique. These membranes are characterized by a fine surface layer compact containing pore or not (called skin or to player) responsible for the selectivity, supported by a porous structure which ensures the mechanical resistance to skin and offers little resistance to transportation. As the thickness of the skin is very thin (less than 1μm), resistance to transport through such membranes is low compared to other dense homogeneous membranes prepared from the same material [3].

In MSP often are used polymeric membranes and polyamide 66 (PA66), one of the polymers used for this application [4]. These are mainly obtained by the technique of phase inversion, since this technique allows controlling important variables that govern the morphology of these membranes thus improving process performance [5-6].

A variety of separation membranes are available on the market today for various applications. Distinct separation processes require membranes with different characteristics. In microfiltration and ultra filtration processes where filtration occurs by size difference, porous membranes that act as sieves are used. Processes such as gas separation and pervaporation, where the transport mechanism and selectivity, involves gas diffusion and solubility, require dense membranes.

*Author a: Centro de Ciências Exatas e Tecnologias, Universidade de Caxias do Sul, Caxias do Sul, Brazil. e-mail: *mzandrad@ucs.br*

© 2016 Global Journals Inc. (US)

In the literature there is a large number of works addressing studies both of the thermodynamics of polymer solutions, as kinetics of mass transfer, formation of the filter skin and the sublayer of the polymeric asymmetric membranes [7].

Knowledge of the thermodynamic properties of a ternary system is relevant to understand and predict the membranes formation [8]. Many researchers have studied the morphology and properties of polyamide membranes [4,9–11] as well as the physicochemical interactions between components in the polymer solution and thermodynamic properties non-solvent (1) / solvent (2) / polymer (3) systems[12–15].

The study of the behavior of polymer solutions require a thermodynamic approach exhibit different aspects of those usually employed in the study of solutions formed by only low molecular mass components [16]. The size and shape of the macromolecules introduce changes in the thermodynamic behavior as well, show large deviations from ideality. From this, it is possible to relate the solubility properties of the components forming part of the system to obtain PA66 in formic acid and/or hydrochloric acid membranes, using water as non-solvent.

a) True Solutions of Polymers

i. *Specific Properties of True Solutions*

According to Tager[17], a true solution is a multi component system which has the following characteristic features: (a) affinity between components; (b) spontaneous formation; (c) constant concentration in time; (d) homogeneity, i.e., presence of only one phase e (e) thermodynamic stability.

Second Lucas et al.[16], the components may mix in any proportion (complete miscibility) or in definite proportions (partial miscibility). If the external conditions (pressure and/or temperature) do not vary, the true solution concentration remains constant in time. A true solution is a system consisting of a single phase. The solution phase may be separated from the other phases (precipitate, vapor or solution) by an interface, but within the solution itself there is no interface, i.e., the system is homogeneous.

The most important feature of a true solution is its thermodynamic stability or equilibrium state. A system is called thermodynamically stable if its formation is accompanied by a decrease in the Helmholtz free energy (at constant volume and temperature) or by a decrease in the Gibbs free energy - ΔG (at constant pressure and temperature). The Gibbs free energy decreases to a definite equilibrium value which does not change subsequently with time[16-17].

ii. *Dissolution and Swelling of Polymers*

According to Schuld & Wolf [18], a polymer does not dissolve in all liquids. In some cases, the polymer and the low molecular liquid (solvent) possess mutual affinity, while in others no such affinity exists. In the former case, the formation of a true solution may be expected, but, in the latter case only a colloidal solution can be formed. The polymer solutions formed spontaneously have all the characteristics of true solutions, including reversibility, equilibrium, swelling before dissolving, high viscosity and slow diffusion. All these features are due to the great difference in size between the particles of the components mixed [19].

Second Canevarolo[20], before dissolving the polymer "swells", i.e., absorbs the low molecular liquid, increasing in mass and volume. Swelling inevitably involves a change in polymer structure, which sharply increases the volume of the samples. Swelling may be limited (it is interaction of the polymer with a low molecular liquid in which the polymer chains are not completely separate from each other) or unlimited (where dissolution occurs spontaneously).

For Tager[17], the degree of swelling can be determined only for polymers with limited swelling, because upon unlimited swelling the polymer itself begins to dissolve and the mass of the sample decreases. Sometimes a phenomenon called negative swelling is observed in polymers, involving decrease rather than increase of sample mass in time.

Polymers may absorb low molecular substances not only from the liquid, but also from the gaseous phase; if the polymer swells on a liquid, it will swell on its vapors as well[16].

Describe the influence of thermodynamic properties in water / hydrochloric acid (HCℓ) / polyamide 66 (PA66) ternary systems with the morphology of the PA66 membranes; starting by characterization of PA66 in HCℓsolutions has motivated this work.

II. Experimental

a) Materials

The polymer used for the solutions and membranes preparation it is the polyamide 66 (PA66) commercial supplied by Alfa Chem. Co.(Brazil), in pellets.

The solvents used in the solutions and membranes preparation were fuming hydrochloric acid (HCℓ) with purity of 37-38% and formic acid (FA) with purity 98-100%, both supplied by Merck (Brazil).

The non-solvent used in the membranes preparation was distilled water. The information of the PA66 and the solvent properties are listed in Table 1.

© 2016 Global Journals Inc. (US)

Table 1: Characteristic data of the solvents and polymer investigated

Materials	Molar volume ($cm^3 \cdot mol^{-1}$)	Molar mass ($g \cdot mol^{-1}$)	Density ($g \cdot cm^{-3}$)
PA 66	10,642.20	11,600[a]	1.22[b] e 1.09[c]
HCℓ (37-38%)	30.67	36.5	1.19
FA (98-100%)	37.7	46	1.22

a = viscosimetric average molar mass (M_v)
b = PA66 density solid (arranged)
c = PA66 density in solution (amorphous)
Source: BRANDRUP & IMMERGUT, 1989

b) Methods

PA66 was dried for 2 hours at 90°C prior to each procedure performed [21].

i. *Preparation of asymmetric flat sheet membranes*

To obtain asymmetric flat sheet membranes two different casting solutions were prepared: PA66 / formic acid / water and PA66 / hydrochloric acid / water, by the phase inversion method(PI) [2].

PA66/HCℓand PA66/FA solutions were prepared using 20 g of PA66 dissolved in 100 cm^3 of each solvent for both systems, at controlled room temperature 25 ± 2°C and at atmospheric pressure (1 atm), under magnetic stirring for 2h. The magnetic stirrer was isolated with glass wool.

The homogeneous casting solutions were cast on glass plate with a knife casting of approximately 200 µm, after was taken solvent evaporation under controlled temperature depending of the solvent used, then immersed in the coagulation bath. The membranes preparation conditions are shown in Table 2.

Table 2 : PA66 Membrane spreparation conditions

	Evaporation conditions		PI* bath conditions	
Membranes	Time (mim)	Temperature (°C)	Time (mim)	Temperature (°C)
FA	10	20	120	25±2
HCℓ	60	60	120	25±2

**PI = phase inversion*

The prepared membranes were washed with distilled water to remove residual solvent and maintained at temperature of 25 ± 2°C for drying. The membranes were called as FA and HCℓ, according use of formic or hydrochloric acid respectively, and them were dried for 24 h at 60°C and characterized by Scanning Electron Microscopy (SEM).

ii. *Scanning Electron Microscopy (SEM)*

Analysis by Scanning Electron Microscopy (SEM) of the surface and the cross section of the membranes were performed on Shimadzu Model SSX550 microscope. Samples were fractured in N_2 liquid at –196°C and metalized by means of a sputtering with a thin gold layer prior to its characterization.

iii. *Solutions preparation and techniques to prove be the polyamide 66 / hydrochloric acid system a true solution*

a. *Affinity between components*

To observe the affinity between components, a solution was prepared using PA66 (20 g) and placed in flat-bottomed flask, to which was added 100 cm^3 hydrochloric acid (HCℓ).

b. *Spontaneous formation*

In order to demonstrate do not be necessary the use of heat or other external force for dissolving the polymer, PA66 (20 g) solution was prepared and placed in double-jacket reactor to which was added 100 cm^3 of hydrochloric acid (HCℓ). In the inner part of the double-jacket was adapted a thermocouple to impart the bath temperature. In contact with the solution, a thermometer measured the temperature of the same during the dissolution process. The system was coupled in a thermostatic bath (25°C).

This experiment was also performed under the same conditions, but with isolated system with glass wool and vacuum in the double-jacket to prevent the heat transfer between the system and the environment.

c. *Constant concentration in time*

To investigate the precipitation possibility (phase separation) of the solution by temperature, PA66 (20 g) solution in 100 cm^3 HCℓ, was frozen in liquid nitrogen(-196°C). Once frozen, the solution was allowed to stand at controlled room temperature (25 ± 2°C) and, when liquid again placed under stirring.

PA66 solutionin HCℓ was placed in double-jacket reactor coupled to temperature controller at -5°C (Frigomix), under magnetic stirring for 2h. After this time, the temperature was raised to 96°C, still under stirring.

d. *Homogenity*

An aliquot of PA66/HCℓ [PA66 (20 g) in 100 cm^3 HCℓ]solution, was placed on a glass coverslip and examined under an optical microscope Carl Zeiss

© 2016 Global Journals Inc. (US)

AxioScope A1 to analyze the presence of interface within the solution.

e. Thermodynamic stability

For to measurethe most important feature of a true solution that is its thermodynamic stability or equilibrium state[16-17], for the PA66/HCℓ and PA66/FAbinary systems, the Gibbs free energy of mixing(ΔG_m) was calculated by the following equation (1):

$$\frac{\Delta G_m}{RT} = x_1 ln\varphi_1 + x_2 ln\varphi_2 + g_{12}(\varphi)x_1\varphi_2 \qquad (1)$$

where φeχ are volume fraction and molar fraction of components; T is the temperature (K) e R is the gas constant. The g_{12} parameter can be considered a free energy term containing contributions enthalpy and entropy[18]. The Gibbs free energy of mixing was calculated as a function of volume fraction of polymer for both solvents.

iv. *Determination degree of swelling*

In order to determine the degree of swelling of PA66, were placed 100 cm^3 of solvent (HCℓ) in flat-bottomed flask. In the mouth flask was adapted polyester fabric, with enough closed mesh to allow the passage of solvent vapor. On the screen a few PA66 pellets was placed. The flask was kept under magnetic stirring for 5h. This method was adapted from Tager[17].

III. Results and Discussion

a) Polymer solution

i. *Specific Properties - True Polymer Solutions*

According to Papkov e Iovleva[22], all systems can be divided into five classes (true solutions containing predominantly solvent; plasticized systems; gels; systems with complete phase separation and polymer dispersions) that comprise polymer with low molecular liquid systems, ranging from true solutions to polymer dispersions in liquids. The low molecular liquid terminology is adopted for solvents, thinners, non-solvents, plasticizers and liquid dispersion media.

Following the concepts of Papkov and Iovleva[22], formic acid (FA) is considered a low molecular liquid (46 $g{\cdot}mol^{-1}$) when compared with polyamide 66 (11,600 $g{\cdot}mol^{-1}$), this system (PA66/FA) is considered by Lin et al. [9] as a true solution.

In order to check if the PA66/HCℓ system displays all the characteristics of true solution such as: affinity between components; spontaneous formation; constant concentration in time; homogeneity and thermodynamic stability, several experiments were performed.

ii. *Affinity between components with spontaneous formation of solution*

To prove affinity between components PA66/HCℓ, the polyamide 66 (PA66) was placed in contact with hydrochloric acid (HCℓ)(Figure 1a)for 2h under magnetic stirring, and the polymer molecules absorbed the solvent, the polymer chains diffused slowly, acquiring mobility and making the homogeneous solution, as can be seen in Figure 1b.

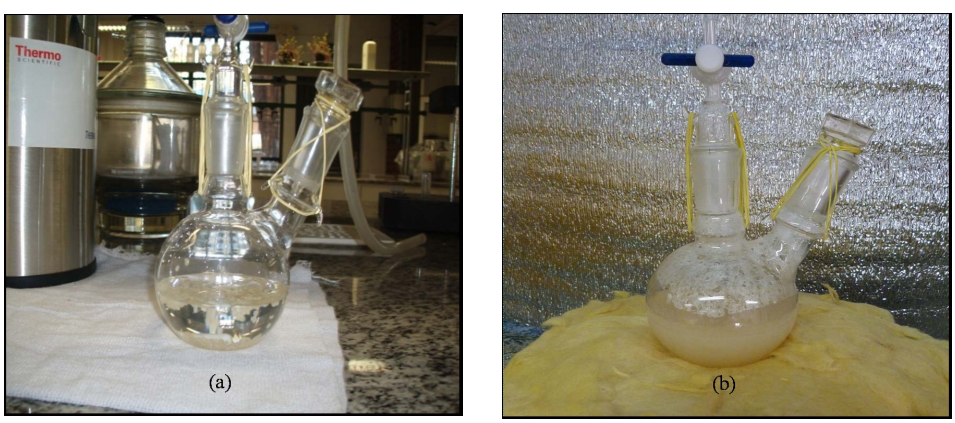


Figure 1: Homogeneous solution of PA66 in HCℓ after testing affinity between components with spontaneous solution formation

According to Tager[17], form true solutions when there is affinity, i.e., there is interaction between components; them disperse spontaneously in direct contact, without the use of heat and/or pressure. Second Lucas et al. [16] this is because the chemical potential of the pure solvent (μ_0) is larger than the chemical potential of the solvent in the solution (μ_1), and when the thermodynamic equilibrium is reached ($\Delta\mu = 0$ or $= \mu_1$) the solution becomes homogeneous. This is corroborated by Gundert & Wolf [23] who claim that HCℓ and FA are good solvents for polyamide at room temperature and atmospheric pressure.

© 2016 Global Journals Inc. (US)

iii. *Constant concentration in time and homogeneity*

Also according Tager[17], if the external conditions (pressure and temperature) do not vary, the concentration of a true solution remains constant over time. In studies by Mulder [2], in most cases, polymer/solvent solutions are characterized by an upper critical solution temperature (UCST), or when cooled (T_0 to T_1) induce phase separation, altering the concentration.

From tests carried out it was observed that when the PA66/HCℓ solution was frozen and thawed, there was no phase separation, the solution remained homogeneous without the presence of precipitates or interface within the solution, as can be seen in Figure 2, only with the presence of air bubbles due to magnetic stirring.

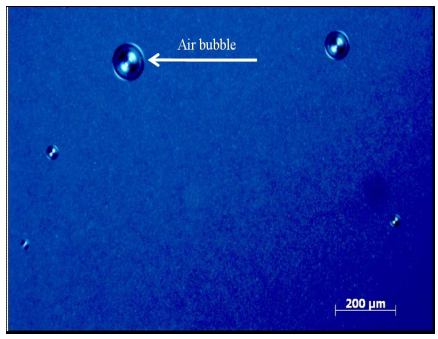


Figure 2: Optical microscopy of the PA66/HCℓ solution - 20 g/100 cm³ (10x)

iv. *Thermodynamic stability*

Second Tager[17]and Lucas et al. [16], a system is called thermodynamically stable if their formation is accompanied by a decrease in the Gibbs free energy of mixing - ΔG_m (constant pressure and temperature), which reduces to a definite value of equilibrium which does not change with time.

So that PA66/HCℓ and PA66/FA binary systems form a homogeneous system (one phase), the Gibbs free energy is negative, as can be seen in Figure 3.The Gibbs free energy of mixing was calculated as a function of volume fraction of polymer for both solvents. The PA66/HCℓ system indicates be more favorable in all concentrations compared to PA66/FA system as values obtained fromΔG_m.

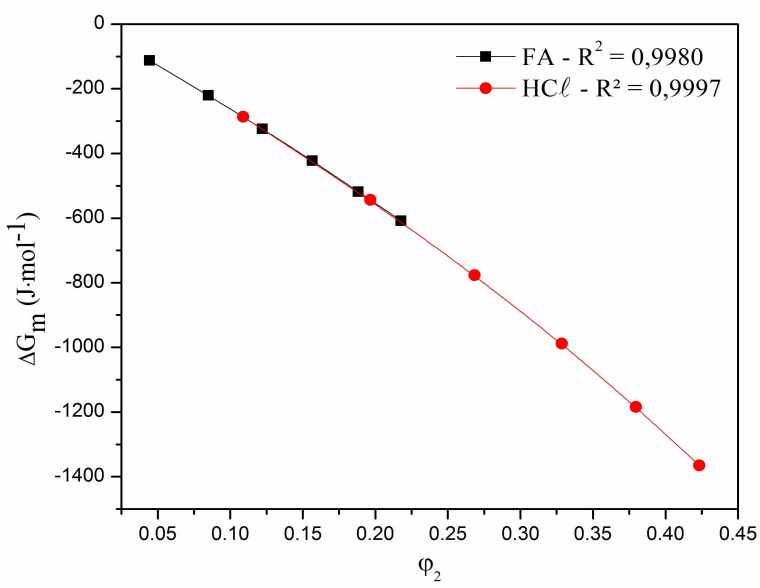


Figure 3: Gibbs free energy of mixing (ΔGm) for PA66/FA and PA66/ HCℓbinary systems as a function of volume fraction of PA66 (φ_2), which is different between systems depending on the concentration of the solvents

The HCℓ (37-38%) is a solution and does not a pure solvent, indicating the presence of water in the PA66 solution, while FA is purity solvent with 98-100%. In the PA66/FA system occur hydrogen bonds between polymer and formic acid, which explains the Gibbs free energy to be negative; in the PA66/HCℓ system dipole-dipole interactions occur, which are also strong interactions. What likely explain the energy of mixing PA66/HCℓ be more negative with respect to PA66/FA system is the acid strength (Ka = acidity constant).

© 2016 Global Journals Inc. (US)

While the HCℓ Ka = 1 x 10^{7}[24-25], formic acid Ka = 1, 77 x 10^{-4}[25], justifying the similar behavior to the polyelectrolyte solution[26-27].

v. *Dissolution and swelling degree of polymers*

According to results obtained for the specific properties of true solutions of the PA66/HCℓ system, confirmed that this is a polymer with low molecular liquid system (HCℓ = 36, 5 g·mol^{-1}) and also is it a true solution, such as PA66/FA system [9]. Being PA66/HCℓ spontaneous system, requiring no external force (pressure and/or temperature) to dissolve the polymer, it was investigated the swelling degree, and according to Lucas et al. [16], if the polymer swells in contact with the low-molecular liquid, also swell in contact with its vapor. The Figure 4 shows the swelling test of the PA66pellets in contact with HCℓ vapor.

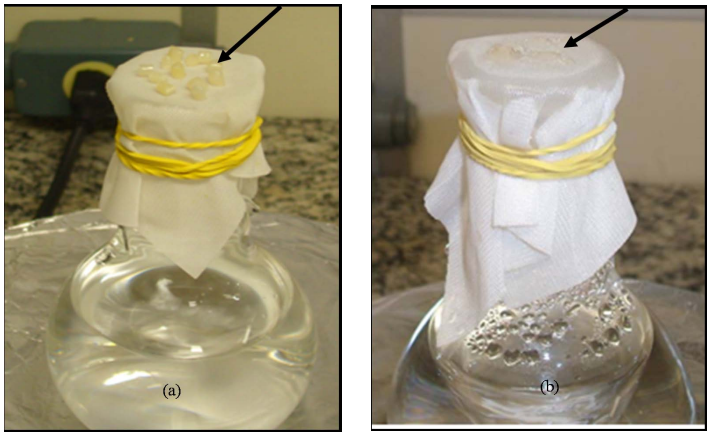


Figure 4 : Dissolution test of the PA66pellets in contact with HCℓ vapor: (a) before and (b) after the test

After 5h the PA66 in contact with HCℓ vapor showed unlimited swelling, i.e. dissolved before swell (Figure 4b). Tager[17] attributed this behavior to interaction between polymer/solvent and solvent strength.

b) Morphological analysis by Scanning Electron Microscopy (SEM) of PA66films

The surface and cross section of the PA66 films were analyzed by scanning electron microscopy (SEM).

The upper surfaces of the films showed dense layer, nonporous, homogeneous and composed of polygonal grains, as can be seen in Figure 5.

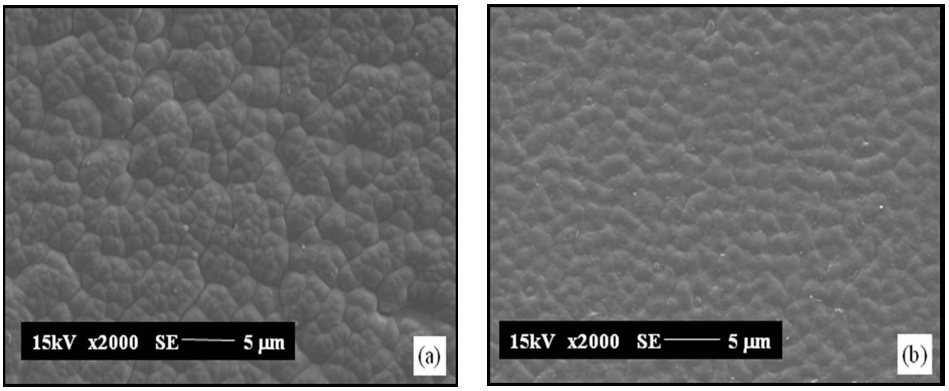


Figure 5: Micrograph (SEM) of surface of PA66 membranes in the form of films prepared in FA (a) and prepared in HCℓ (b) (2000x)

Shih et al.[28]and Lin et al.[9], also studied the mechanisms of phase separation on PA66 membranes in FA and, this polygonal characteristic with limits almost linear are similar to spherulites, that are obtained for phase separation solid-liquid (S-L) (crystallization).

The morphology of the PA66films in formic acid and hydrochloric acid is typical of polyamide films precipitate on considered aggressive bath (water), with dense layer and cellular and pore structure. The micrograph (Figure 6a) shows a PA66film in FA with a dense layer of approximately 4 µmthickness and pores cell small surrounded by a polymer matrix. The micrograph (Figure 6b) shows a PA66 film in HCℓ with dense layer of approximately 23 µm in thickness and

© 2016 Global Journals Inc. (US)

also has pores cell small surrounded by a polymer matrix.

Figure 6 shows the cross section of the PA66 films in FA and HCℓ which present similar morphologies.

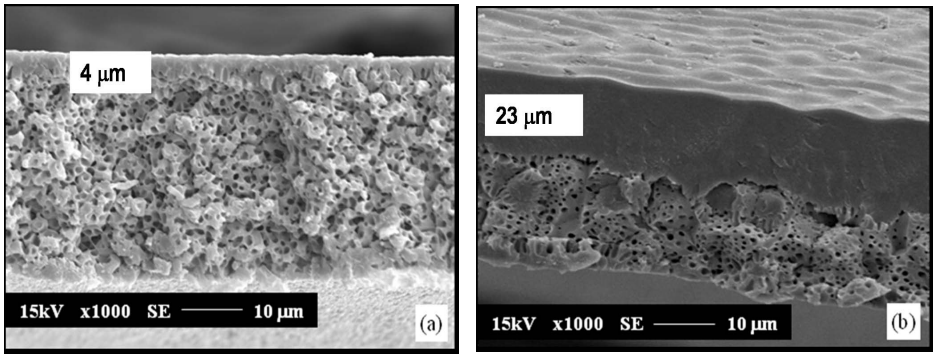


Figure 6: Micrograph(SEM) of the cross section of the PA66 films in: FA (a) and HCℓ (b) (1000X)

Looking at Figure 6 of the standpoint of Lin et al. [9], the non-porous dense layer formation is due to the increased concentration of polymer in the film interface with the bath (water). During precipitation a thick gel layer was formed on the surface excluding the possibility of nucleation liquid micelles in the region (pore formation). This process was more intense in PA66/HCℓ films, but these films were subjected to a solvent evaporation step, where according Mulder [2] is the beginning of precipitation, prior to the immersion bath. This probably favored the polymer concentration highest on the surface, resulting in increased thickness of the dense layer (23 µm) in relation to PA66/FA films (4 µm).

IV. Conclusions

The solution of PA66 in FA is considered a true solution in the literature, however, to solution PA66 in HCℓ was not found reference. The specific properties analyzed proved that the solution of PA66 in HCℓ behaves as a true solution, including the final morphology of the membranes that are similar membranes obtained by binary system PA66/FA.

V. Acknowledgements

The authors thank Caxias do Sul University (UCS), Lecturer Dr. Otávio Bianchi (UCS) and CNPq for support in this work.

References Références Referencias

1. F.J. Bassetti, Preparação, caracterização e aplicação de membranas poliméricas microporosas assimétricas, Universidade Estadual de Campinas, 2002.
2. M. Mulder, Basic Principles of Membrane Technology, second, Kluwer Academic Publ, London, 2003.
3. R.W. Baker, Membrane Technology and Applications, John Wiley & Sons Ltd, California, 2004.
4. C.W. Yao, R.P. Burford, A.G. Fane, C.J.D. Fell, Effect of coagulation conditions on structure and properties of membranes from aliphatic polyamides, J. Memb. Sci. 38 (1988) 113–125. doi:10.1016/S0376-7388(00)80874-1.
5. K. Scott, Handbook of industrial membranes, Elsevier Advanced Tecnology, Oxford, 1995.
6. R.N. A.C. Habert, C.P. Borges, Processos de separação com membranas, E-papers, Rio de Janeiro, 2006. http://www.e-papers.com.br.
7. J.C.C. Petrus, Preparation, modification and characterization of asymmetric membranes for clarification of fruit juice, Universidade de Campinas, 1997.
8. A.M.W. Bulte, E.M. Naafs, F. van Eeten, M.H.V. Mulder, C.A. Smolders, H. Strathmann, Equilibrium thermodynamics of the ternary membrane-forming system nylon, formic acid and water, Polymer (Guildf). 37 (1996) 1647–1655. doi:10.1016/0032-3861(96)83714-1.
9. D.J. Lin, C.L. Chang, C.K. Lee, L.P. Cheng, Fine structure and crystallinity of porous Nylon 66 membranes prepared by phase inversion in the water/formic acid/Nylon 66 system, Eur. Polym. J. 42 (2006) 356–367. doi:10.1016/j.eurpolymj.2005.07.007.
10. M. Zeni, R. Riveros, J.F. de Souza, K. Mello, C. Meireles, G.R. Filho, Morphologic analysis of porous polyamide 6,6 membranes prepared by phase inversion, Desalination. 221 (2008) 294–297. doi:10.1016/j.desal.2007.03.012.
11. P. Poletto, J. Duarte, M.B. Thürmer, V. dos Santos, M. Zeni, Characterization of Polyamide 66 membranes prepared by phase inversion using formic acid and hydrochloric acid such as solvents, Mater. Res. 14 (2011) 547–551. doi:10.1590/S1516-14392011005000087.
12. F.W. Altena, C.A. Smolders, Calculation of liquid-liquid phase separation in a ternary system of a polymer in a mixture of a solvent and a nonsolvent,

© 2016 Global Journals Inc. (US)

Macromolecules. 15 (1982) 1491–1497. doi:10.1021/ma00234a008.
13. R.M. Boom, H.W. Reinders, H.H.W. Rolevink, T. van den Boomgaard, C.A. Smolders, Equilibrium Thermodynamics of a Quaternary Membrane-Forming System with Two Polymers. 2. Experiments, Macromolecules. 27 (1994) 2041–2044. doi:10.1021/ma00086a010.
14. A.M. Bulte, M.H.V. Mulder, C.A. Smolders, H. Strathmann, Diffusion induced phase separation with crystallizable nylons. II. Relation to final membrane morphology, J. Memb. Sci. 121 (1996) 37–49. doi:10.1016/0376-7388(96)00163-9.
15. J. Zhang, Y. Zhang, J. Zhao, Thermodynamic study of non-solvent/dimethyl sulfoxide/polyacrylonitrile ternary systems: effects of the non-solvent species, Polym. Bull. 67 (2011) 1073–1089. doi:10.1007/s00289-011-0525-9.
16. E.F. Lucas, B.G. Soares, E. Monteiro, Caracterização de polímeros: Determinação de Peso Molecular e Análise Térmica, Editora E-papers, Rio de Janeiro, 2001. http://www.e-papers.com.br.
17. A. Tager, Physical Chemistry of Polymers, Second ed., Mir Publishers, Moscow, 1978.
18. N. Schuld, B.A. Wolf, Polymer–solvent interaction parameter, in: J. Brandrup, E.H. Immergut, E.A. Grulke (Eds.), Polym. Handb., 4th ed., John Wiley & Sons, Inc., New York, 1999.
19. L.H. Sperling, Introduction to Physical Polymer Science, 4th - ed, John Wiley & Sons, Inc., New Jersey, 2006.
20. Jr.S.V. Canevarolo, Ciência dos polímeros: um texto básico para tecnólogos e engenheiros, Artliber, São Paulo, 2010.
21. J. Brandrup, E.H. Immergut, Polymer Handbook, 3rd- ed., John Wiley & Sons, Inc., New York, 1989.
22. S.P. Papkov, M.M. Iovleva, Classification of polymer-low molecular liquid systems according to their physical condition, Vysok. Soyed. 16-3(1974) 614–619.doi:10.1016/0032-3950(74)90373-6.
23. F. Gundert, B.A. Wolf, Polymer–solvent Interaction Parameter, in: J. Bramdrup, E. H. Immergut (Eds.), Polym. Handb., 3rd ed., John Wiley & Sons, Inc., New York, 1989.
24. Y. V Moiseev, Chemical Resistance of Polymers in Aggressive Media, Plenum, New York, 1987. http://books.google.com.br/books?id=8N2fzAFw18C&pg=PA30&lpg=PA30&dq=basicity+constant+of+the+polymer&source=bl&ots=lGlOxfATal&sig=nzBp9ReoaCiQuBlSSm_v3NWllek&hl=ptBR&sa=X&ei=Q21OT_32Kcm_gQfSjpHxBA&sqi=2&ved=0CH4Q6AEwCA#v=onepage&q=basicity constant of the.
25. D.R. Lide, The CRC Handbook of Chemistry and Physics, 76th - ed, CRC Press, Boca Raton, 1995.http://chemwiki.ucdavis.edu/Organic_Chemistry/Organic_Chemistry_With_a_BiologicalEmphasis/Reference_Tables/Representative_acid_constants.
26. L.P. Cheng, A.H. Dwan, C.C. Gryte, Isothermal phase behavior of Nylon-6,-66, and-610 polyamides in formic acid–water systems, J. Polym. Sci. Part B Polym. Phys. 32 (1994) 1183–1190. http://onlinelibrary.wiley.com/doi/10.1002/polb.1994.090320706/abstract (accessed August 22, 2012).
27. J. Duarte, Análise termodinâmica do sistema ternário água/poliamida 66/ácido clorídrico, Caxias do Sul, 2013. http://www.ucs.br.
28. C.H. Shih, C.C. Gryte, L.P. Cheng, Morphology of membranes formed by the isothermal precipitation of polyamide solutions from water/formic acid systems, J. Appl. Polym. Sci. 96 (2005) 944–960. doi:10.1002/app.21545.

© 2016 Global Journals Inc. (US)

Global Journal of Science Frontier Research: B
Chemistry
Volume 16 Issue 2 Version 1.0 Year 2016
Type : Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Field and Laboratory Studies on Four Species of Sea Squirts and their Larvae

By Gaber Ahmed Saad & Abdullah Bedeer Hussein
Dammam University, Saudi Arabia

Abstract- The aim of this study was to characterize adult distribution with respect to light and analyze ovary contents in the four seasons of the year. The swimming behavior of *Ciona intestinalis*, *Molgula manhattensis*, *Ascidella aspersa* and *Phallusia mammilata* larvae against certain abiotic factors were commented. For the field data on adult distributions, one-way analysis of variance (ANOVA) was applied to test for differences in adult orientation, with surface orientation as a fixed factor. Two adult species (*Ciona intestinalis* and *Molgula manhattensis*) showed no orientation with respect to light while in the other two species (*Ascidella aspersa* and *Phallusia mammilata*) light exerted a significant effects on the orientation and density of individuals. To evaluate among the different species the level of gregariousness found in the field, the number of individuals per clump for each species has been compared using one-way ANOVA, with species as a fixed factor. Artificial heterologous inseminations were carried out.

Keywords: field data - gregariousness - heterologous inseminations -adult orientation - larval settlement – phototaxis - geotaxis.

GJSFR-B Classification : FOR Code: 069999

FIELDANDLABORATORYSTUDIESONFOURSPECIESOFSEASQUIRTSANDTHEIRLARVAE

Strictly as per the compliance and regulations of :

© 2016. Gaber Ahmed Saad & Abdullah Bedeer Hussein. This is a research/review paper, distributed under the terms of the Creative Commons Attribution-Noncommercial 3.0 Unported License http://creativecommons.org/licenses/by-nc/3.0/), permitting all non commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Field and Laboratory Studies on Four Species of Sea Squirts and their Larvae

Gaber Ahmed Saad [α] & Abdullah Bedeer Hussein [σ]

Abstract- The aim of this study was to characterize adult distribution with respect to light and analyze ovary contents in the four seasons of the year. The swimming behavior of *Ciona intestinalis*, *Molgula manhattensis*, *Ascidella aspersa* and *Phallusia mammilata* larvae against certain abiotic factors were commented. For the field data on adult distributions, one-way analysis of variance (ANOVA) was applied to test for differences in adult orientation, with surface orientation as a fixed factor. Two adult species (*Ciona intestinalis* and *Molgula manhattensis*) showed no orientation with respect to light while in the other two species (*Ascidella aspersa* and *Phallusia mammilata*) light exerted a significant effects on the orientation and density of individuals. To evaluate among the different species the level of gregariousness found in the field, the number of individuals per clump for each species has been compared using one-way ANOVA, with species as a fixed factor. Artificial heterologous inseminations were carried out. Three experiments were investigated in the laboratory on metamorphosed larvae of the four species. The first experiment tested geotaxis with respect to phototaxis during larval settlement. One species (*Ciona intestinalis*) showed a clear preference for settlement on top surfaces Top > Lateral = Bottom whereas the three species (*Molgula manhattensis*, *Ascidella aspersa* and *Phallusia mammilata*) settled on the bottom than elsewhere Bottom > Lateral = Top. In the second experiment, larvae were placed in wells in which half of the top, bottom or lateral surfaces were covered by black tape while the other half of these surfaces were exposed to light. Few number of larvae in one species preferred the top regardless of light direction dark = light while three species showed a significant interaction between light/ darkness and position, showing a marked preference for dark surfaces and bottom orientation. Larvae of two species continued to prefer bottom surface in the light but selected both bottom and top in the dark. One species changed light preferences depending on the surface considered, The third experiment tested the effect of adult mantle extract on larval settlement. One species (*Ascidella aspersa*) showed little effect of mantle tissues extract while the other three species (*Ciona intestinalis*, *Molgula manhattensis* and *Phallusia mammilata*) showed a significant inhibition of settlement.

Keywords: field data - gregariousness - heterologous inseminations -adult orientation - larval settlement – phototaxis - geotaxis.

Author α: Department of Biology, Deanship of preparatory year and supporting studies, Dammam University, Saudi Arabia, KSA, Department of Zoology, Faculty of Science, Alexandria University, Alexandria, Egypt. e-mail: gaibrahim@uod.edu.sa

Author σ: Department of Biology, Deanship of preparatory year and supporting studies, Dammam University, Saudi Arabia, KSA, Department of Zoology, Faculty of Science, Mansoura University, Mansoura, Egypt.

I. Introduction

The metamorphosis of the ascidian tadpole larvae has long attracted the interest of many authors. Using tadpoles of *Ciona* , *Phallusia*, *Ascidia* ,*Styela*, *Styelopsis*, *Distomus*, *Clavelina* and *Distaplia*, Berrill (1947 a & b) ; Byrne et al. (2003) concluded that ascidians spread only during larval stage and acid metabolites produced by the activity of the tail were the inducing agent of metamophosis. Moreover, two factors were identified during the metamorphosis of the ascidian tadpole, namely 'aging factor' which is the progressive exhaustion of the yolk reserves of the epidermis. The epidermis, muscles and notochord are affected. 'Nutritive exhaustion' culminates in the centripetal contraction of the epidermal envelope, a contraction that appeared to have a disruptive effect upon the tissues within (Underwood and Keough 2001). It was mentioned that adult ascidians are capable of slow-crawling (Hecht, et al. 1977; Goodbody & Fisher1974 ; Goodbody &Gibson1974). This movement involved the progressive formation of new colonies for attachment. Through light microscopy examination, Lester(1988) investigated the process of settlement and metamophosis of *Rhabdopleura nomani* (Hemichordata: Pterobranchia). These colonies were collected from Brmuda, USA. He concluded that the swimming larvae settle within 24 hrs. In the work of Cloney (1961), it was mentioned that the resorption of the tail of *Boltenia vilosa*, *Pyura haustorlo* and *Styela gibbsii* began proximally and progresses distally. The sheath at the anterior end of the notochord ruptured at the beginning of tail resorptuon. The matrix of notochord flowed into the body cavity of the trunk. The muscle cells shorten and buckle as the tail shortened and the myofibrills of these cells become disarranged. Gilbert and Raunio (1997) mentioned that there are many reasons of why ascidians are popular as research animals in developmental biology. In fact, ascidian embryos and larvae have a small number of cells, at the beginning of gastrulation, ascidian embryos contain only about 110 cells whereas amphibian gastrulae contain about10 000 cells. Ascidian tadpole larva consists of a few thousand cells and only six different tissues. Ascidian larvae develop rapidly. Swimming larvae hatched after 12-18 hrs post fertilization. Some ascidians have eggs with colored cytoplasmic regions. These regions distribute during cleavage to specific blastomeres. This feature

© 2016 Global Journals Inc. (US)

allows cell fates to be followed by routine microscopy. Ascidians may provide basic information about the development of more complex chordates such as vertebrates. Ascidians have small genomes that facilitate cloning genes involved in developmental process from another point of view, there are some limitations in using ascidians as an experimental system. This is due to the fact that most of them have restricted breeding seasons and living embryos can be obtained only at certain times of the year. Moreover, genetic analysis is not available as it is in *Drosophila* for example. However, genetic approaches have been developed in some of the compound ascidians (Rinkevich and Weissman (1987). It is well-known that ascidians live solitary or colonial. In the text-books such as by Young, et al. (1988), Satoh (1994), and Gilbert & Raunio (1997), the solitary forms are commonly named 'Ascidiae simplicies' whereas those forming colonies are named 'Ascidiae compositae'. In the work of Niermann-Kerkenberg(1989), ascidians in general are referred to as 'mantle animals'.

The length of the swimming period in oviparous species can range from only few hours to several days. While in most viviparous species, it ranges from a few minutes to several hours (Byrne et al. 2003; Bullard et al. 2004). The larva swims for 6 hrs. and prepare for the onset of metamorphosis. It alters its response to light and gravity. The larva is first negative geotactic and positive phototactic. Immediately before settlement it avoided light and prefers to settle on dark or shaded surfaces. According to Cloney (1982 &1990; Hinz & Schwarzlaender 2004), the principal structures of solitary ascidian larvae are classified into 3 groups: transistory larval organs; prospective juvenile organs and larval juvenile organs. The complexities and degree of differentiation of these components vary for different taxa (Millar,1971; Hinz and Schwarzlaender, 2004). Metamorphosis involved numerous rapid morphogenitic movements and physiological changes that are initiated at the moment of settlement (Gilbert &Raunio,1997; Campbell & Donlan 2005; Dupont et al. 2006). Transistory larval organs are phagocytized or otherwise destroyed at metamorphosis whereas the prospective juvenile organs and larval-juvenile organs become the functional parts of the juvenile or oozooid. Metamorphosis began with settlement and is followed by series of coordinated morphogenetic movements that rearrange cells, tissues and organs (Calvo-Ugarteburu & McQuaid 1998; Kasper et al. 2008 ; Ross & Auge 2008; Rius et al. 2009a). The axial complex of the tail, the visceral ganglion and the sensory organs of the cerebral vesicle are destroyed and engulfed by phagocytes. The presence of adults and associated chemical factors is normally regarded as an attractor for settlement alongside conspecific adults (Koh & Sweatman 2000; Ramsay et al. 2008) or an inducer of metamorphosis (Svane et al. 1987 ; Lambert 2005, 2007; Whiteley & Bendell-Young 2007), which may cause aggregation. Phototactic and/or geotactic behavior of the larvae can determine where settlement occurs (Svane and Young 1989). For all these reasons, settlement has the capacity to influence habitat selection, determining adult distribution patterns of sedentary species (Underwood & Keough 2001). Cloney (1990) listed 10 principal events of ascidian metamorphosis: Secretion of adhesives by papillae or the epidermis of the trunk; Eversion and retraction of papillae; Resorption of the tail; Loss of the outer cuticular layer of the larval tunic; Emigration of blood cells or pigment cells; Rotation of visceral organs through an arc of about 90 degrees; Expansion of the branchial basket and elongation of the oozooid or juvenile; Expansion, elongation or reciprocation of ampullae; Reorientation of test vesicles, and expansion of the tunic; Retraction of the sensory vesicle; Phagocytosis of visceral ganglion, sensory organs and cells of the axial complex and Release of organ rudiments from an arrested state of development. The same author added that metamorphosis may be completed in seconds or minutes (papillary eversion and tail resorption) and others may take hours or days (rotation, ampullar outgrowth, phagocytosis of the axial complex. Ascidians are major contributors (Lambert 2005, 2007), and can severely modify the structure of coastal habitats by forming large aggregates (Lambert & Lambert 2003, Rius et al. 2009a). Adults live attached to hard substrata (Monniot et al. 2001) and the only motile stage is their lecithotrophic larvae, which have very limited dispersal due to their short planktonic lifespans (Svane & Young 1989). Some information is available regarding the distribution of adult ascidians in the field (Mastrototaro et al. 2008), although the settlement patterns that may explain these adult distributions are well-understood for only a few species (Svane & Young, 1989; Valiela et al., 1997; Howes et al. 2007). Many factors can influence ascidian larval behavior and settlement, including light, gravity, temperature, salinity, presence of adults or competitors, biomechanical properties and energy limitations (Svane & Young 1989; Stachowicz et al. 1999; McHenry & Patek 2004; Bennet & Marshall 2005). There has been a rapid increase in the rate of introduced non-indigenous ascidian species to many parts of the world in the past 20–40 years (Lambert, 2001). Changes in seawater temperatures due to global climate change over the last 25 years may be facilitating this spread (Young & Chia 1985; Stachowicz et al., 2002). It was noticed that the number of the individuals in the field varied according to the season and hence the number of the specimens obtained in every collection varied too. There found also seasonal variations in the size of these animals. It was observed that very small-sized specimens may be less than 5 mm in length appeared during the period from March to September.

© 2016 Global Journals Inc. (US)

This study investigated the settlement patterns of larvae of four solitary ascidians found along the estuarine water of the Arabian Gulf (Saudi Arabia) namely *Ciona intestinalis* (Linnaeus, 1767), *Molgula manhattensis*, (De Kay, 1843), *Ascidella aspersa* (Müller,1776) and *Phallusia mammilata* (Cuvier,1815), which belong to different families from the enterogoneate ascidians (Kott, 1985) and are all commonly found aggregated in the field (Rius et al. 2009a, Branch et al. 2010, and personal observation). These species were chosen to include introduced species with global distributions (*Molgula manhattensis*, *Ascidella aspersa*) and two large native species (*Ciona intestinalis* and *Phallusia mammilata*) that are not known to be invasive, although congeners are recognized as invasive elsewhere (Castilla et al. 2004). All these species are important occupiers of hard substrata of coastal areas of Arabian Gulf (Branch et al. 2010). The larvae of all species had well developed statocytes and ocelli (Niermann-Kerkenberg, 1989; Lübbering, 1994; Kriegel, 1996; Hofmann, et al. 1999; Jacobs et al. 2008 and personal observation) but *Ciona intestinalis* has a highly reduced ocellus (Ohtsuki 1990), and *Molgula manhattensis* is unusual among Pyuridae in lacking an ocellus (personal observation, and see also Svane & Young 1991 for a closely related species). Thus, three species were expected to have both light and geotactic preferences, while the larvae of the remaining species were expected to respond to geotactic stimuli alone. How larval behavior determines settlement patterns in different phototactic and geotactic conditions was examined and in the presence or absence of conspecific extracts. The larval responses were compared with patterns of adult distribution in the field. Three specific hypotheses were advanced. (1) Light will influence settlement, with dark being preferred over light in species that are found in dark habitats, and the opposite for those that occur in well-lit habitats. (2) Geotactic behavior will be important in those species that have adults with clear orientation preferences. (3) Adult mantle tissue extract will have a positive effect on settlement on all the species, and will contribute to the aggregated patterns of distribution of adults.

II. Material and Methods

a) Field Study

Adult specimens of *Ciona intestinalis* (Linnaeus, 1767), *Molgula manhattensis* (De Kay, 1843), *Ascidella aspersa* (Müller,1776) and *Phallusia mammilata* (Cuvier,1815) were surveyed and sampled from four estuarine locations of the Arabian Gulf namely Al-Azezia, Southern Khobar. Northern Khobar and Ad-Dammam. They were transferred into aquaria containing see water and microorganisms to the laboratory. Immediately they are transferred to 10 liter-plastic aquaria with fresh filtered sea water, perfect aeration has been continuously carried out and suspensions of Microcell® were administrated as food. Sea water has been changed every other day. Aquaria were kept at temprature18 Ċ. At each location adult distribution and associated circumstances were quantified. To standardize conditions, all sampling took place at 12:00 am on cloudless days in January/December 2013 at depths of no more than 1 m. At each locality, 50x50 cm quadrats (n=10 per substratum orientation) were placed on horizontal hard substrata facing upwards (0-10°), downwards (170-180°), or on vertical substrata (80-100°). The number of individuals of any of the four species present and the number of individuals per clump were counted. Due to the aggregating nature of ascidians and because they were often covered by algae or other fouling organisms, Clumps were removed and brought them to the laboratory where they could be cleaned and sorted to count the number of individuals precisely. Light intensity was recorded at each sampling point by taking three random measurements within each quadrate using a photometer (Skye 177 instruments Ltd, Scientific Associates) fitted with a sensor (Quantum Sensor, Wales).

b) Laboratory Study

All laboratory experiments were conducted during the early spring of 2013 (end of August to early September) to coincide with the timing of reproductive maturity for all species: *Ascidella aspersa* and *Phallusia mammilata* mature in spring and summer (Rius et al. 2009a), *Ciona intestinalis* and *Molgula manhattensis* in spring, summer and winter (Rius et al. 2009b; Hofmann, et al. 2008 ; Saad, et al. 2010 & Saad, 2002), previous observations undertaken before and after this study to conform some results and observations.

c) Artificial heterologous insemination (Fertilization assays)

About 10 adults of each species were collected from each of the locations specified and transported in insulated containers with 20 Litre seawater to the laboratory within five hours. In the laboratory, specimens were housed in aerated seawater and maintained at room temperature (20°C). All manipulations and experiments were undertaken in filtered seawater obtained using vacuum filtration through 10-µm pore-size filters. For *Ciona intestinalis* and *Ascidella aspersa*, artificial fertilization followed the methods of Young & Chia (1985) and Hofmann, et al. 2008), which involved dissection and collection of gametes from the oviduct and sperm duct. For the remaining two species the methods of Marshall et al. (2000), modified from those of Svane & Young (1991): gametes were extracted by dissection of the ripe gonads and a mix of eggs and sperm poured through a 100-µm filter with seawater into a small beaker, so the eggs were retained by the filter, but the excess sperm and seawater passed through into the beaker. For all species cross of gametes of four

© 2016 Global Journals Inc. (US)

individuals, preventing self-fertilization. Developing embryos were placed in an aerated beaker (containing 500 ml seawater) in a constant-temperature cabinet at 20°C and complete darkness. In all species, motile larvae hatched within 14 h of fertilization.

d) Experimentations

The experimental units were transparent cylindrical Perspex containers, sealed at the top and bottom with Perspex sheets and held together with an elastic band. The cylinders were 11 mm tall and 44 mm diameter with exactly the same surface area (15.205 cm^2) on the top, bottom, and lateral surfaces, thus offering equivalent surface areas for larval settlement in each of these three orientations. The containers were placed in a seawater tank for 24 hours prior to introduction of larvae, to create a biofilm, which is known to enhance settlement (Keough & Raimondi 1995). Once motile larvae of a given species were formed, they were pipetted out and placed 20 larvae per container filled with seawater (final volume 16.72 cm^3), and immersed the containers in seawater in a 200 ml beaker at 20°C for 24 hours under the experimental conditions detailed below. The Perspex chamber was subsequently dismantled in seawater, so that any unattached larvae were washed away. Three experiments were performed. The number of replicates (i.e. experimental units with 20 larvae each) per treatment and experiment varied from 3 to 10 due to variability in the number of larvae obtained. Once enough larvae were obtained in a given fertilization event, All the experiments described below in were ran parallel. The first experiment involved exposing the wells with larvae to either artificial light (47 μ mol m-2 s-1) or complete darkness (0 μ mol m-2 s-1). In the second experiment, which was modified from the approach of Jiang et al. (2005), larvae were placed in wells in which half of the top, bottom and lateral surfaces were covered by black tape (reducing the light to 0.4 μ mol m-2 s-1), while the other half of these surfaces were exposed to the same artificial light (47 μ mol m-2 s-1).

The third experiment tested the effect of adult extracts on larval settlement, and for this the general method of Svane et al. (1987) was followed, which involved dissolving mantle tissues extract in seawater. An initial concentration of 0.5 g (wet weight) of tunic, previously homogenised using a blender and filtered to eliminate the biggest fragments, was diluted in seawater to obtain a final concentration of 5 % in the experimental wells. Settlement of larvae in seawater with or without mantle tissues extract (control treatment) was then compared in complete darkness. In all three experiments, a stereomicroscope was used to count the numbers of settlers and score their orientation (top, bottom or lateral sides of the containers) after a 24-hour period.

e) Laboratory investigations on gonadal overview of Phallusia mammilata Macroscopic observation

Three specimens for each species from the seasonally collection, (the size of each species is the same), were dissected, the tunic was incised from dorsal side and entirely removed, the mantle was opened dorsally, the branchial sac was excised to expose properly the reproductive system. A part of the hermaphrodite gonad was separated. All preparations were fixed in 10% formalin.

f) Microscopic observation

The same preparations of the macroscopic ones were washed in distilled water for 24 hours, dehydration through ascending series of ethyl alcohol, followed by another dehydration series of tertiary butyl alcohol, tertiary butanol and paraffin oil (1:1), absolute paraffin oil. All preparations then washed in tissue mate or paraplast with melting point 54-58 Ċ and blocked in fresh paraplast. Sections of 5-8 μ were more or less obtained.

One specimen from each species, as a whole mount preparation, was treated like the last preparations to be examined histologically trying to investigate the relationship of varies tissues of the body to the reproductive system. The two siphons for each animal were cut to minimize the length of the specimen from one side and to obtain better embedding. A Number of stains were tried to enable differentiation of the different stages of oocyte and testicular follicles. These are Eherlich haematoxylin and eosin (Pantin,1948); Heidenhain's iron haematoxylin (Pantin,1948); Mallory triple stain (Pantin,1948); Masson's trichrome stain (Pantin,1948); Weighert's haematoxylin & Van-Gieson stain (Mahoney,1975); 6. Alcian blue countered with eosin (Pearse,1968).

Statistically the number of the previtellogenic; vitellogenic; postvitellogenic and atretic oocytes were counted in three ovarian lobules for each histological preparation of four investigated ascidian species in all seasons of the year.

g) Statistical analysis

Analysis of variance (ANOVA) is a broad group of calibrations for identifying and measuring different sources of variation within the data set. It consists of a set of procedures by which a variance of the random variable is broken down by certain sources of variation of its value. With the components of variance, depending on the sources, one can conclude if there is a significant difference between the values of dependent variable for different levels of the observed factor variables. In the present study, a one-way analysis of variance is used to compare significant differences among different orientations. To evaluate among the different species the level of gregariousness found in the field, the number of individuals per clump found for each species with species as a fixed factor and adult

© 2016 Global Journals Inc. (US)

orientation, surface orientation was used as a fixed factor. The number of settlers was tabulated and analyzed incorporating replicates (experimental wells), treatments (light - dark, extract-control) and position of the settlers (bottom, lateral, top). If the above-mentioned assumptions for ANOVA are not met, the Turkey`s Multiple Comparison Test, Bartlett's test for equal variances and Dunnett's Multiple Comparison Test were used for determining whether three or more independent samples originate give a clear cut differences. When this test leads to significant results, at 1 North one sample differs from the others. A principal component analysis is a standard tool in modern data analysis. It is a simple, nonparametric method for extracting relevant information out of confusing data sets. Principal component analysis is concerned with the interpretation of the variance and covariance structure of the original set of variables through a small number of their linear combinations. The general objectives of principal component analysis are data reduction and interpretation. In order to reduce the number of variables. For more details about methodology of calibrations. However, One-way analysis of variance (ANOVA) at $P<0.0001$, Bartlett's test for equal variances, Tukey's Multiple Comparison Test, Dunnett's Multiple Comparison Test and Newman-Keuls Multiple Comparison Test at $P > 0.05$ and $P < 0.001$ were applied (Knoke and Burke 1991; Quinn and Keough 2002 ; Systat Inc., 2007 ; Dijana, et al.2012 for review).

III. Results

Solitary ascidians of this study produce a large number of eggs that are around 150 μm in ϕ. Forms with external fertilization like most phlebobranchs and stolidobranchs spawn small, rather simple sperm. Sperm from solitary ascidians can swim for extended periods and undergo hypermotility in the vicinity of conspecific eggs. In addition, ascidian eggs release factors that increase the motility of sperm and can cause the directed swimming of the sperm to the egg. The embryos of ascidians can be obtained in unlimited quantity by artificial fertilization. It is sufficient to incise an adult from the right side, cut across the oviduct and vas deferens, having previously noticed that the former contained ova, and then collect the ova and sperms as they pass out from the point of incision with a glass tube, and transfer them to a glass containing fresh sea water, and mix them well, but gently, together by stirring. Gradually the ova sink to the bottom of the glass, and in about an hour after the above operation they commence to embryogenesis. The successive cleavage furrows were described. Due to the equal distribution of the yolk granules, the egg undergoes an equal holoblastic cleavage. The first cleavage furrow is meridional resulting in 2 equal and blastomeres (after 2 hrs post fertilization. The second cleavage furrow is also meridional but perpendicular to the first one resulting in 4 blastomeres (after 2:15 hours post fertilization). The third cleavage furrow is latitudinal. It transects the first and second furrows medially resulting in 8 blastomeres (after 2:30 hours of fertilization). The fourth cleavage furrow is two meridional divisions at the same time resulting in 16 blastomeres (after 3:40 hours of fertilization). The fifth cleavage furrow is two latitudinal divisions at the same time. One in the animal hemisphere and the other in the vegitative one resulting in 32 blastomeres (after 3:41till 4 hours of fertilization). After the fifth cleave furrow, this process becomes complicated and perhaps two or three or more furrows occur at the same time. However the 64-blastomere stage was observed. The blastomeres gradually get smaller in size forming a compact sphere. After cleavage the resulting blastomeres moved to the periphery where they arrange themselves forming a continuous lamina or blastoderm. The latter encloses a concentric cavity or blastocoel. This stage of development is referred to as blastula stage (After 4 hrs. post fertilization). The blastomeres at one pole of this sphere, the vegetative pole, flatten and begin to invaginate. This invagination proceeded transforming the single celled-layer blastula into a double cell-layered gastrula (After 4:30 hrs post fertilization). Thus a new cavity appeared which increases gradually in size on the expense of the size of the segmentation cavity. The new cavity formed is referred to as gastrocoel which has an aperture opening, the atriopore.

The blastomeres surround the archentric cavity are the cells of mesendoderm while those surrounding the segmentation cavity are the ectodermal cells.

At the dorsal side of the gastrula precisely in its median portion the ectodermal cells flattens forming the so-called neural plate then invaginate. This invagination proceeded forming the neural cavity which is bordered with two neural ridges. The two neural ridges come close to each other then unite transforming the neural cavity into a neural tube with a central neurocoel and this stage represents the neurula (After 7 hrs post fertilization).

Organogenesis then begins after about 9 hours and finally the embryo inside its chorion is distinguished into an oval trunk and a long tail after 11 hours.

Hatching of the larva takes place by hatching enzyme (After 12 hours of fertilization). Three stages of development of the investigated ascidians were collected from the sea water using a micropipete and all were fixed afterwards in 4 % paraformaldhye and described. Stage 1: This larva has a long tail and an oval trunk. It swims actively and very quickly. It swims at the superficial stratum of the sea water and does not escape from the field of microscope. In other words the larva at this stage is negatively geotactic and positively phototactic. The outer covering of the larva is somewhat transparent. The trunk has two adhesive papillae at its

© 2016 Global Journals Inc. (US)

proximal end, internally the alimentary tract is a simple tube having an ingestion opening and an egestion one.These two openings orifice on the dorsal side of the trunk. The nervous system is represented only by a brain vesicle having a ventrally situated statolith and a small otolith. This vesicle has an opening referred to as neuropore according to Müller and Hassel (1999.). This neuropore opens in the alimentary tract through neuroentric canal. The brain vesicle extends posteriorly in the tail region as a nerve cord with its central canal. According to Tannenbaum and Rosenbluth (1972), there are a myoneural junctions between the muscle cells and the nerve cord. This cord bears an eye at its junction with the brain vesicle. Torrence and Cloney (1975) illustrated perfectly the fine sensory neurons in the adhesive papillae in the larvae of ascidians and Tannenbaum & Rosenbluth (1972) interrested in the study of the myoneural junctions in larval ascidian tail, Underneath the nerve cord runs a rod-like notochord consisting of vacuolated cells.In addition to these structures, the trunk contains also some mesenchyme cells which later differentiate into tissue or organ rudiments. The tail is muscular and it appears morphologically that the muscle fibres are of striated type (according Mackie & Bone, 1976). There is an undulated fin covering the tail from its dorsal and ventral sides. The test cells were previously entirely embedded in the cytoplasm of the oocyte while after hatching they evaginated and cover this larval stage from all directions especially in case of the larvae of *Ascidiella aspersa* (Fig. 1). Stage II: The trunk gets larger in size. The process of phagocytosis of the tail starts in the direction from posterior to anterior. Torsion of the alimentary tract begins. At this stage of development the nervous system is still represented by the brain vesicle and the test cells decrease in number together with thickening of the outer covering of the larva. This larval stage swims always near the bottom of the sea water and escape from the field of microscope. In other words it is positively geotactic and negatively phototactic (Fig. 2). Stage III: After complete phagocytosis and resorption of the tail, the young metamorphosed ascidian is in the form of the trunk of the larval stadium. At this stage three important criteria can be observed: The tunic is already formed but still somewhat transparent with complete disappearance of the test cells from around it. Rotation of the GI is terminated. The ingestion and the egestion openings of the larva in stage II larval have distinguished into a mouth and an anus respectively. The mouth presents on the oral siphon and the anus presents on the atrial siphon. Closure of the neuropore and the brain ventricle of the larval stage now after metamorphosis differentiates into a nerve ganglion and a neural gland dorsally located to the former (Fig. 3).

a) Adult distribution

Each of the species examined exhibited differences in habitat orientation in the field (Histogram1). The morphology and the anatomy of adults and their embryology of *Ciona intestinalis*, *Ascidella aspersa*, *Phallusia mammilata* and other species were previously studied (Hofmann, et al. 2008; Saad, et al. 2002 & 2010). In both artificial and natural substrata, *Ciona intestinalis* and *Molgula manhattensis* were most abundant on illuminating surfaces, while *Ascidella aspersa* and *Phallusia mammilata* individuals densities were found in poorly illuminating surfaces. Orientation had significant effects on the density of individuals (Table 1) (ANOVA, *Ciona intestinalis*, $F = 6{,}847$, $p < 0.0001$, Tukey test, $p < 0.05$, Upwards > Downward, both = Vertical; *Molgula manhattensis*, $F = 14{,}07$, $p < 0.0001$, Tukey test, $p < 0.01$, Downwards > Upwards and Vertical; *Ascidella aspersa*, $F = 9{,}025$, $p = 0{,}0001$, Tukey test, $p < 0.05$, Upwards > Downwards, both = Vertical. In the case of *Phallusia mammilata*, $F = 13{,}16$, $p < 0.0001$ no significant differences among orientations was found. *Ciona intestinalis* showed Bartlett's statistic (corrected) =23,06 and R squared =6,847 and was most abundant on downward-facing surfaces, and both *Molgula manhattensis* and *Ascidella aspersa* were more abundant on downward and vertical surfaces with Bartlett's statistic (corrected) ranges from zero to 20,97 and R squared =0, 5751 - 0, 6896. *Phallusia mammilata* $F = 13{,}16$, $p < 0.0001$, Tukey test, $p < 0.05$, Upownwards > Downwards, both = Vertical, Bartlett's statistic (corrected) =30,03 and R squared =0,6638. Light intensities were usually highest on vertical surfaces (Histogram 1) due to the characteristics of the floating panels from where the animals were collected, Except for *Molgula manhattensis*, the only species collected from iron platforms and bottom of ships in study shore localities. Low light intensities on upward-facing surfaces for the remaining species reflected the fact that they grew on artificial substrata (panels) that were poorly illuminated due to other structures that screened them.

b) Effects of light and orientation on larval settlement

In the first experiment, results for *Ciona intestinalis* and *Molgula manhattensis* showed that settlers have an obvious difference between darkness and illumination. For the remaining species, *Ascidella aspersa* and *Phallusia mammilata* there was significant interaction of the light with the position of the settlers (Histogram 2 and Table 2). When the two factors were analyzed separately, no effect of the light/dark treatment was found (Histogram 2, Table 2 and *t*-tests on proportion of settlers: all $p > 0.05$). For the position factor, *Ciona intestinalis* showed a clear preference for settlement on top surfaces Top > Lateral = Bottom, proven through Log-likelihood and LR Chi-Square where 122.300, 2.561 and 0.369 represent top, lateral and

© 2016 Global Journals Inc. (US)

bottom positions respectively, whereas the three species *Molgula manhattensis*, *Ascidella aspersa* and *Phallusia mammilata* settled significantly more often on the bottom than elsewhere Bottom > Lateral = Top (Histogram 2, Table 2).

In the second experiment, in which the larvae had the option of settling on light or dark surfaces in the same chamber, a different picture emerged (Table 3, Histogram 3). Few number of larvae preferred top position in case of *Ciona intestinalis* regardless of light direction dark = light.

For *Molgula manhattensis*, *Ascidella aspersa* and *Phallusia mammilata*, significant interaction was found between treatment and position, showing a marked preference for dark surfaces, and bottom orientation. It was found also that larvae of *Ascidella aspersa* and *Phallusia mammilata* continued to prefer bottom surfaces in the light but selected both bottom and top in the dark. *Molgula manhattensis* changed light preferences depending on the surface considered, but overall more larvae settled in light, and it preferred lateral surfaces in the illuminating part of the wells. These results are generally in accordance with what found in the field for adults of *Ciona intestinalis*, *Ascidella aspersa and Phallusia mammilata* (Histogram 1), all of which settled in the dark, and also for *Ascidella aspersa*, which (largely) settled in the light. The four species that displayed significant geotactic patterns in the first experiment shifted to a more random pattern in the second experiment, with two (*Ciona intestinalis* and *Molgula manhattensis*) now showing no geotactic preferences, and the other two species (*Ascidella aspersa and Phallusia mammilata*) showing greater settlement on lateral and top surfaces than previously. When the two factors are considered in the experiment (position of larval settlement and direction of light), darkness enhance larvae of *Molgula manhattensis, Ascidella aspersa and Phallusia mammilata* to prefer settlement on the bottom where bottom > lateral = top but *Ciona intestinalis* showed no significant results for this experiment.

c) Effect of mantle tissues extract

Ascidella aspersa showed little effect of mantle tissues extract in the water (Histogram 4, Table 4 and Turkey`s Multiple Comparison tests, $p > 0.05$). The other three species *Ciona intestinalis*, *Molgula manhattensis* and *Phallusia mammilata* showed a significant inhibition of settlement in the presence of mantle tissues extract, no extract > extract (Histogram 4, Table 4, and Turkey`s Multiple Comparison tests, all $p < 0.05$). Although in *Ciona intestinalis* the log-linear analysis revealed a significant interaction, with the extract inhibition being significant for the lateral and top surfaces only. The geotactic behavior found in the first experiment testing light/dark effects was maintained across all species in this third experiment, with the three *Molgula manhattensis*, *Ascidella aspersa* and *Phallusia mammilata* settling preferentially on the bottom (Histogram 4). For *Ciona intestinalis*, the highest number of settlers was again on top surfaces, although in the presence of adult extract there was no significant difference between top and bottom (Table 4). For *Ascidella aspersa* there was no position effects, and for *Phallusia mammilata* there was no effect of either extract or position on settlement in the wells.

d) Integrating field and laboratory data

Comparing the level of aggregation and the overall abundance of individuals in the field (Histograms 1 - 5 & Tables 1 - 5), a consistent pattern emerged: the more abundant a species was in a particular orientation, the more individuals there were per clump. When analyzed, the number of individuals per clump across species, *Phallusia mammilata* showed the highest numbers (Histogram 5), but significant differences existed only between *Molgula manhattensis* and two other species *Ciona intestinalis* and *Ascidella aspersa* (ANOVA, $F = 8.075$, $p = 0.0084$, Turkey`s Multiple Comparison tests, Tukey test, *Molgula manhattensis* > *Ascidella aspersa* = *Ciona intestinalis*, $p < 0.05$). In terms of the numbers of individuals per clump in relation to orientation in the field (Histogram 5), significant differences emerged for two species (ANOVA, *Ciona intestinalis*, $F = 0.9956$, $p = 0.0802$, Turkey`s Multiple Comparison tests, Upwards greater than the other two orientations, $p < 0.05$; *Phallusia mammilata*, $F = 18.00$, $p < 0.0001$, Turkey`s Multiple Comparison tests, Downward greater than the other two orientations, $p < 0.001$).

For an overall perspective of the geotactic preference of each species, settlement data generated from the three laboratory experiments were pooled together , on the assumption that in terms of geotactic behavior, larvae in the field would encounter a combination of both phototactic stimuli and adult extracts. Setting aside *Ciona intestinalis* and *Ascidella aspersa* on the grounds that their settlement rates were too low for consideration, the mean percentage of settlers on each surface showed the same trend as the number of individuals per clump for three species (*Molgula manhattensis*; *Ascidella aspersa* and *Phallusia mammilata*), whereas *Ciona intestinalis* showed no correlation (Histogram 5).

Three trends emerged from the laboratory data (as summarised in Table 5). First, in relation to orientation, one species (*Ciona intestinalis*) tended to settle preferentially on the top, whereas three (*Molgula manhattensis; Ascidella aspersa and Phallusia mammilata*) preferred settling on the bottom in experiment 1, with almost the same pattern emerging in experiment 3. In experiment 2 the geotactic responses evident in experiment 1 were either absent or altered. *Molgula manhattensis* and *Phallusia mammilata* could be

© 2016 Global Journals Inc. (US)

analyzed with respect to geotactic behavior only in experiment 3, and neither showed any preference. Second, in terms of light/dark responses, none of the four species analyzed showed any statistical preferences in experiment 1, where the larvae were held either in light or dark. However, in experiment 2, when they had a choice between dark and light, three species (*Molgula manhattensis*, *Ascidella aspersa* and *Phallusia mammilata*) displayed preference for settling in the dark, and a fourth (*Ciona intestinalis*) settled most often in the light, although this preference changed on bottom surfaces, leading to an interaction between the factors.

Third, in relation to the presence or absence of adult mantle tissues extract in the third experiment, two species (*Ascidella aspersa* and *Phallusia mammilata*) showed no response of mantle tissues extract in the water, while settlement of the other two species (*Ciona intestinalis and Molgula manhattensis*) was inhibited in the presence of mantle tissues extract.

e) Laboratory observation on the gonad of the adult stage of Phallusia mammilata

The single hermaphrodite gonad is present inside the intestinal loop (Fig. 4). The ovary appeared massive with yellowish-orange tinge in all specimens dissected in the different seasons of the year. It extended anteriorly along the dorsal side of the animal as long oviduct which runs parallel to the intestine and terminated inside the atrial cavity. The macroscopic observation of the ovary did not show any details. As the gonad was longitudinally and very superficially incised at its narrower end, a milky yellowish-colored inclusion release and this represents the sperm suspension but when incised deeper at its wider end, the oocytes are seen and appeared to be arranged linearly parallel to each other being crowded in the central lumen of the ovary. The macroscopic observations revealed that the gonad is sac-like structure which is more wider at its distal extremity and narrows gradually near the rectum then join a narrow gonoduct which was orange in color and full of rounded masses of large oocytes. These large masses of oocytes are always with orange tinge having peripheral nuclei and with dark ooplasm. The testis in gross anatomy is over-looked, however, testicular diverticulae can be observed on the outer wall of the stomach and intestine. At the anterior apex of the stomach the gonad was represented only by the testis. This male part is branched and ramified into several testicular diverticulae. They were attached on the outer wall of the stomach and surrounded with the outer atrial wall being dominant and in excess inside the intestinal loop. The germinal epithelium of all diverticulae is in continuation as a single lamina. Underneath the simple cubical germinal epithelium the different stages of spermatozoa development could be seen. Along the dorsal side of the animal and parallel to the rectum these branches of testis join each other forming a common vas deferens which open in the atrial cavity. The ovary is a saccular structure lied under the testis and more posteriorly oriented. It is surrounded with the outer atrial wall externally. The ovary in the macroscopic observation seemed to be one part but histologically the germinal epithelium marked the external boundary of the ovary and gave off internal branches that unite each other (Fig. 4). The germinal epithelium divided the ovary internally into lobules. In each lobule the different stages of oocyte development are observable. Both the sperm duct and oviduct are much longer and have no relation with the mantle. The vas deferens attached along the intestine and the oviduct lied on its top. Different seminiferous tubules (testicular diverticulae) of the same animal showed different histological appearance i.e., the process of spermatogenesis and spermeogenesis (spermeohistogenesis) were well observed in all histological preparations taken in the different seasons of the year. This means that the testis is always mature all the year round. At a high magnification there are some very small rounded cells darkly stained and scattered in between the spermatozoa. They are larger in size than the cells in the spermatid layers. The different stages of oocyte development were described and counted in three ovarian lobules taken from three different squirts in each season of the year.

f) Squirts collected during spring

i. *First ovarian lobule*

Small-sized oocytes (50-70 μm) were arranged along the germinal epithelium (Figs. 5-6). These masses of small oocytes gained blue stain with different histological dyes. The small oocytes near the germinal epithelium are surrounded by very small rounded haemocytes which are randomly scattered inside the matrix of the ovary (Fig. 8). The small oocytes were present in aggregates inside the lobules of the single ovary. The ooplasm was homogenous without granulation and vacuolated, nucleus was pale while nucleolus seemed darkly stained. Each small oocyte was bounded externally with a darkly stained cell membrane and a single layer of follicular epithelium. There was another kind of oocytes scattered randomly inside the lobules (Fig. 9). They are large-sized (80- 100 μm), having dark granulated ooplasm, The small rounded cells around the small oocytes are completely absent in the vicinity of these large ones. These masses of large oocytes are follicular. At the periphery of ooplasm scattered test cells were observed. These large oocytes are always oval-shaped.

There is another type of large follicular oocytes (110-130 μm) having granulation only at the periphery of ooplasm which was darkly stained, while in the central region of ooplasm lacked granulation and this region is pale stained completely similar to the staining affinity of the ooplasm of the small oocytes. The matrix of the

© 2016 Global Journals Inc. (US)

ovary was of connective tissue and blood vessels. See Histogram 6 & Figs. 4-9 for review.

ii. *Second & third ovarian lobules*

The same ovarian appearance of the first preparation but the ovary contains variable number of follicular oocytes. The internal lobulation of the ovary was well-marked. The internal folded germinal epithelium divided the lumen of the ovary into compartments. These internal compartments contained the different stages. Some atretic large-sized oocytes (160 – 180 μm) were observed.

g) Squirts collected during summer

i. *First & second ovarian lobules*

Small oocytes (50 - 70 μm) were arranged at the periphery of the ovary near the germinal epithelium and each one was surrounded externally with small rounded cells. Each small oocyte was bounded with a dark cell membrane, ooplasm was pale stained, homogenous without granulation but vacuolated, nucleus was pale while the nucleolus was darkly stained. Large follicular oocytes (170 - 180 μm) were observable (Fig. 10). They were distributed randomly inside the ovary and having dark granulated ooplasm and chorion. The outer follicular squamous cells were elevated over the inner follicular cells. The nucleus was pale while the nucleolus was darkly stained. Another type of oocytes can be seen inside the ovary. This oocyte was larger in size, oval-shaped and completely decayed ruptured inclusions inside, without definite outer membrane (Fig. 11). It seems to be undergone autolysis. The matrix of the ovary was more homogenous than that of the ovary of squirts in the preceding period. See Histogram 6 & Figs. 10-11 for review.

ii. *Third ovarian lobule*

Very small rounded oocytes (30 -50 μm) were observable attached to the germinal epithelium. Moderately-sized oocytes (80 -100 μm) were scattered randomly inside the lobules of the ovary and they were surrounded externally with very small cells. In this preparation , large-sized masses of oocytes were observed. These oocytes were rounded and rarely oval shaped. The ooplasm was dark stained and strongly granulated. The granules were darker than the ooplasm itself. At the periphery of each oocyte there were a dark chorion, a pervitelline space, test cells arranged at the periphery of cytoplasm, an inner and an outer follicular epithelia, The nucleus was always pale while the nucleolus is darkly stained. Atretic large-sized oocytes were present. They haven't a definite shape, their outer follicular epithelium was discontinuous, Moreover inside the ooplasm many vacuoles were present. The matrix was crowded with connective tissue, haemocytes and decayed contents.

h) Squirts collected during autumn

i. *First ovarian lobule*

The lobulated ovary was compact and completely full of oocytes. Very small oocytes presented near the germinal epithelium surrounded with very small cells. Moderately-sized oocytes were distributed randomly. Large follicular oocytes were found and distributed randomly. Atretic oocytes are in excess. The matrix was full of connective tissue, small cells surround the small and moderately-sized oocytes (haemocytes) and decayed contents. See Histogram 6 & Figs. 4-11 for review.

ii. *Second & third ovarian lobules*

The lobulated ovary was compact and full of oocytes at different stages of development. The only difference between these two ovaries and these described in the first preparation was that the germinal epithelium here was stretched because of the huge amount of oocytes inside the ovary.

i) Squirts collected during winter

i. *First, second & third ovarian lobules*

Small-sized oocytes were rare near the germinal epithelium. Full follicular oocytes (170 – 180 μm) were distributed randomly inside the matrix. Each small or follicular oocyte was surrounded with very small-sized cells (haemocytes), rounded or oval follicular oocytes were present in excess. Atretic large-sized oocytes were observable. See Histogram 6 & Figs. 4-11 for review.

IV. Discussion

Many benthic marine invertebrates reproduce by releasing sperm into the sea (free-spawning), but the amount of time that sperm are viable after spawning may have different consequences for fertilization, depending on the type of free-spawner. In egg-broadcasting marine organisms, gamete age is usually assumed to be irrelevant because of the low probability of contact between dilute sperm and egg. However, direct dilution effects might be reduced in egg-brooding free-spawners that filter dilute sperm out of the water column, and sperm longevity may play a role in facilitating fertilization in these taxa. To a large extent, the range of conditions where adults of each species occurred in the field correlated well with the behavior of the larvae in the laboratory. *Ciona intestinalis* is a common fouling species in sheltered marine and harbours (Dybern, 1965 ; Monniot et al. 2001; Lambert & Lambert 2003), where it is found in relatively dark places on the lower surfaces of substrata. This study concluded that *Ciona intestinalis* and *Molgula manhattensis* were most abundant on poorly illuminating surfaces, while *Ascidella aspersa* and *Phallusia mammilata* individuals densities were found in poorly illuminating and well illuminating surfaces. Orientation had significant effects on the density of individuals. Moreover, no significant

© 2016 Global Journals Inc. (US)

differences were found among settlement orientations in *Ciona intestinalis*. Correlated with this, its larvae showed preferences to settle on beneath the upper surface of the experimental wells. Both *Molgula manhattensis* and *Ascidella aspersa* were more abundant on downward and vertical surfaces. *Phallusia mammilata* preferred to settle Upownwards > Downwards, both = Vertical. The field study concluded that low light intensities on upward-facing surfaces for the remaining species *Molgula manhattensis*, *Ascidella aspersa* and *Phallusia mammilata* reflected the fact that they grew on artificial substrata (panels) that were poorly illuminated due to other structures that screened them. Light intensities were usually highest on vertical surfaces due to the characteristics of the floating panels from where the animals were collected, *Ciona intestinalis* lives on well-illuminating upper or lateral surfaces and its larvae settled on the bottoms or sides of wells and preferred light conditions when settling on the sides. *Molgula manhattensis, Ascidella aspersa* and *Phallusia mammilata* adults displayed clear preferences for dark surfaces, and accordingly their larvae preferred dark conditions and upward-facing surfaces. *Ciona intestinalis* exhibited no habitat preference in the field and no preferential geotactic or phototactic larval responses. When the phototactic preference for dark places and geotactic behavior in those species with clear orientation preference were supposed, emphasising the importance of settlement in determining adult distribution patterns, with three of the four species displaying larval behavior that was in agreement with field observations. In addition, this study showed how the biotic factor examined (presence or absence of mantle tissues extract) and the two abiotic factors (phototaxis and geotaxis) can play an integrated role in determining settlement patterns, providing insight into how such factors may influence adult distribution in the field. In the first experiment, when larvae were held under either light or dark conditions, geotactic preferences drove larval behavior. However, in the second experiment, when larvae had the option of choosing between shaded and light conditions, three species clearly preferred to settle on dark surfaces. Our results are in accordance with the general statement that shading facilitates the dominance of hard substrata by sessile invertebrates while well-illuminating surfaces lead to algal-dominated communities (Miller and Etter 2008). For those species settling in the dark, this might incidentally lead to settlement among adult conspecifics, where light is reduced in the shade of adults, ultimately contributing to a gregarious distribution.

An interesting result of the second experiment was that few number of settlers on less illuminating surfaces in case of *Ciona intestinalis*. On the contarary, in case of *Molgula manhattensis, Ascidella aspersa* and *Phallusia mammilata*, no significant interaction was found between treatment and position. *Molgula manhattensis* changed light preferences depending on the surface considered, but overall more larvae settled in light, and it preferred lateral surfaces in the illuminating part of the wells. Moreover, the three last species showed a marked preference for dark surfaces, and no significant preference for any orientation. It was found also that larvae of *Ascidella aspersa* and *Phallusia mammilata* continued to prefer bottom surfaces in the light but selected both bottom and top in the dark. These results are generally in accordance with what found in the field for adults of *Ciona intestinalis*, *Ascidella aspersa* and *Phallusia mammilata*, all of which settled in the dark, and also for Ascidella aspersa, which (largely) settled in the light. The four species that displayed significant geotactic patterns in the first experiment shifted to a more random pattern in the second experiment, with two (*Ciona intestinalis* and *Molgula manhattensis*) now showing no geotactic preferences, and the other two species (*Ascidella aspersa* and *Phallusia mammilata*) showing greater settlement on lateral and top surfaces than previously. A considerable observation noticed in the laboratory that larvae of all species altered their geotactic behavior from that displayed in the first experiment, showing a more haphazard geotactic settlement distribution or alteration of preferences in the second experiment. These results contrast with what has previously been found for the tadpole larvae of another solitary ascidian (*Ascidia mentula*) and for the planulae of a scyphozoan, in which the larvae did not alter their negative geotactic behavior across a range of light conditions. This study suggests that during settlement, time of day and weather conditions (which can alter light conditions) may greatly influence larval behavior. Most species of ascidians are invasive (M. Rius, C.L. Griffiths and X. Turon, in preparation) and have succeeded in establishing populations worldwide (Lambert & Lambert 2003; Barros et al. 2009). The fact that there were no settlement preferences in either of these species may indicate that they can successfully settle under a range of conditions and on a range of surfaces, increasing the likelihood of their colonising new localities. Young and Braithwaite (1980) have shown that *Styela montereyensis*, like *Styela plicata* and *Ascdilla aspersa*, shows no discrimination with respect to light or substratum type. Similarly, Young and Chia (1985) failed to find any settlement preferences in six other solitary ascidian species that were exposed to different light regimes. In this study it was found that strong patterns in the terms of light/dark responses, none of the four species analyzed showed any statistical preferences in experiment 1, where the larvae were held either in light or dark. However, in experiment 2, when they had a choice between dark and light, three species (*Molgula manhattensis, Ascidella aspersa* and *Phallusia mammilata*) displayed preference for settling in the dark,

© 2016 Global Journals Inc. (US)

and a fourth (*Ciona intestinalis*) settled most often in the light, although this preference changed on bottom surfaces, leading to an interaction between the factors. In relation to orientation, one species (*Ciona intestinalis*) tended to settle preferentially on the top, whereas three (*Molgula manhattensis; Ascidella aspersa* and *Phallusia mammilata*) preferred settling on the bottom in experiment 1, with almost the same pattern emerging in experiment 3. In experiment 2 the geotactic responses evident in experiment 1 were either absent or altered. *Molgula manhattensis* and *Phallusia mammilata* could be analyzed with respect to geotactic behavior only in experiment 3, and neither showed any preference. Perhaps the ocelli on the trunk play a role in response to light intensities in the ascidian larvae.

This contrasts with the behavior of the larvae of a closely related species that lacks photoreceptors, *M. exasperatus*, which displays no light sensitivity or preferences (Svane & Young 1991). Both conspecific attraction and gregarious behavior have been identified as driving forces for the distribution of many organisms (Alonso et al. 2004; Gautier et al. 2006). This study concluded that in relation to the presence or absence of adult mantle tissues extract in the third experiment, *Ascidella aspersa* showed little effect of mantle tissues extract in the water while the other three species *Ciona intestinalis* , *Molgula manhattensis* and *Phallusia mammilata* showed a significant inhibition of settlement in the presence of mantle tissues extract. Similar to these findings, the percentage of metamorphosis of the solitary ascidian *Molgula citrina* decreases when its larvae are exposed to conspecific tunic homogenate (Dupont, et al. 2006). This has implications for understanding how prior invasions might affect further colonization. This study showed also that settlement was not promoted by the presence of adult extracts. However, it is possible that the adult extracts acted as a repellent because they signaled damaged tissues of a conspecific; but other authors using adult extracts have found that the presence of extracts induced metamorphosis (Svane et al. 1987), so this study has found the same result for two studied species *Ascidella aspersa* and *Phallusia mammilata*. This study suggests that the gregarious distribution of adults observed in the field are unlikely to be explained by larval attraction to adult extracts, but may be the result of settlement being concentrated in habitats characterised by particular physical conditions. For many other marine species, physical factors seem to be stronger factors for settlement than chemical attraction by conspecific adults (Berntsson et al. 2004). Sometimes these preferred physical conditions such as light intensity and hydrodynamic conditions may coincidentally be associated with the presence of adults, or even created by adults, leading indirectly to aggregations. For instance, a baffle effect of created by aggregations of adults (Eckman, 1983) may enhance the settlement of new larvae and protect the juveniles, thereby increasing their survival. However, more needs to be learnt concerning the mechanisms driving the effect of conspecific adult attraction and further experiments using gregarious ascidians have the potential to provide important insights. In confined environments, such as harbours and marine environment, where invasive ascidians are highly successful, the specific biological features of each species such as larval movement and offspring retention, the particular hydrodynamics of the location (Havenhand & Svane 1991) and adequate conditions for settlement (as shown in this study) may play important roles in influencing species distributions and the success of introduced populations. For example, *Ciona intestinalis* is widespread in estuarine conditions in harbours and successfully colonizes the culture ropes of mussel farms, with important economic impacts (Robinson, et al. 2005), as also in northeast American coastal waters (Ramsay, et al. 2008). Biotic and chemical factors, other than those arising from conspecific adults, may determine aggregated settlement of ascidians in the field (Hadfield & Paul 2001). However, results of this study favour the view that the aggregated distribution of the solitary ascidians considered reflects responses to abiotic rather than biotic factors, although there is always the possibility that complex biotic interactions, such as competition or facilitation, occur during juvenile and adult stages, as it has been demonstrated in other gregarious organisms (Rius & McQuaid 2009). There is a need to further study the mechanisms that determine gregarious distribution in invasive species. Comparisons of species performance and biology across both introduced and native ranges could be enlightening (Bossdorf, et al. 2005). Concepts such as conspecific and kinship attraction, and gregarious behavior should be incorporated to the study of the distribution of invasive species, as they might be key features for our understanding of the viability and success of these populations.

Literatures

1. Alonso, J. C. ; Martin, C. A. ; Alonso, J. A.; Palacin, C.; Magana, M. and Lane, S. J. (2004): Distribution dynamics of a great bustard metapopulation throughout a decade: influence of conspecific attraction and recruitment. Biodiversity Conserv., 13:1659-1674.
2. Barros, R. C.; Rocha, R.M. and Pie, M. R. (2009): Human-mediated global dispersion of *Styela plicata* (Tunicata, Ascidiacea). Aquatic Invasions 4:45-57.
3. Bennet. C. E. and Marshall, D. J. (2005): The relative energetic costs of the larval period, larval swimming and metamorphosis for the ascidian *Diplosoma listerianum* Mar. Freshw. Behav. Physiol., 38:21-29.

© 2016 Global Journals Inc. (US)

4. Berntsson, K. M.; Jonsson, P. R.; Larsson, A. I. and Holdt, S. (2004): Rejection of unsuitable substrata as a potential driver of aggregated settlement in the barnacle *Balanus improvisus*. Mar. Ecol. Prog. Ser., 275:199-210.
5. Berrill N. J. and Sheldon H. (1964): The fine structure of the connections between muscle cells in ascidian tadpole larva. J. Cell Biol. 23: 554-669.
6. Berrill, N. J. (1947a): Metamorphosis in ascidians. J. Morph., 80 : 249-267.
7. Berrill, N. J. (1947b): The development and growth of *Ciona.* J. Mar. Biol. Ass., U. K. 26: 616-625.
8. Bishop C.D. , Bates W.R. and Brandhorst B.P. (2001): Regulation of metamorphosis in ascidians involves NO/cGMP signaling and HSP90. J. Exp. Zool. 289: 374-384.
9. Bone Q. and Ryan K. P. (1979) : The Langerhans Receptor of *Oikopleura* Tunicata: Larvacea). J. mar. biol. Ass. U.K. 59: 69-75.
10. Bossdorf, O.; Auge, H.; Lafuma, L.; Rogers, W. E.; Siemann, E. and Prati, D, (2005): Phenotypic and genetic differentiation between native and introduced plant populations. Oecologia, 144:1-11.
11. Branch, G. M.; Griffiths, C. L.; Branch, M. L. and Beckley, L. E. (2010): Two Oceans: A guide to the marine life of southern Africa. Struik Publishers, Cape Town.
12. Bullard, S. G.; Whitlatch, R.; Osman, R. W. (2004): Checking the landing zone: Do invertebrate larvae avoid settling near superior spatial competitors? Mar. Ecol. Prog. Ser., 280:239-247.
13. Byrne, P. G.; Simmons, L. W. and Roberts, J. D. (2003): Sperm competition and the evolution of gamete morphology in frogs. Proc. R. Soc. Lond. B, 270:2079-2086.
14. Calvo-Ugarteburu, G. and McQuaid, C. D. (1998): Parasitism and invasive species: effects of digenetic trematodes on mussels. Mar. Ecol. Prog. Ser., 169:149-163.
15. Campbell, K.and Donlan, C. J. (2005): Feral goat eradications on islands. Conserv. Biol., 19:1362-1374.
16. Castilla, J. C.; Guinez, R.; Caro, A. U. and Ortiz, V. (2004): Invasion of a rocky intertidal shore by the tunicate *Pyura praeputialis* in the Bay of Antofagasta, Chile. Proc. Natl. Acad. Sci., U S A 101:8517-8524.
17. Cloney, R. A. (1961): Observation on the mechanism of the resorption of the tail in ascidians. Am. Zool., 1:67-87.
18. Cloney , R.. A. (1975): Ascidian metamorphosis. Review and analysis. In Chia E. S. andRice M. (eds.). Amesterdam Elserict. North Holland Biomedical Press pp 255-282.
19. Cloney ,R.A. (1982) : Ascidian larvae and the events of metamorphosis. Amer. Zool. 22: 817-826.
20. Cloney , R. A. (1990): Urochordata : Ascidiacea. In K. G. Adiyodi and R. G.Adiyodi (eds.). Reproduction Biology of Urochordata. Vol. IV B. Fertilization, Development and Parental care. New York , Oxford pp 391-451.
21. Dawson A. B. and Hlsaw ,jR F. L. (1964): The Occurrence of Neurosecretory Cells in the Neural Ganglia of Tunicates. J. Morph. 114:411-424.
22. Dijana, G. ; Jasminka, D.; Vesna, S. and Teuta, T. 2012. Analysis of Heavy Metals concentration in Wastewater along Highways in Croatia. J. Computing Info. Technol., CIT 20(3):209–215.
23. Dhargalkar, V. K. and Verlecar, X. N. 2004. Zooplankton Methodology, Collection & Identification. S.C. Goswami (Retd.). National Institute of Oceanography Dona Paula, Goa - 403 004. Ministry of Environment & Forests, New Delhi. http://www.researchgate.net/publication/237447982_Zooplankton_Methodology_Collection__Identification
24. Dilly N. (1961): Electron Microscope observation of the receptors in the sensory vesicle of the Ascidian tadpole. Nature, 191:786-787.
25. Dupont, L.; Richard, J.; Paulet, Y. M. ; Thouzeau, G. and Viard, F. (2006): Gregariousness and protandry promote reproductive insurance in the invasive gastropod *Crepidula fornicata*: evidence from assignment of larval paternity. Mol. Ecol., 15:3009-3021.
26. Dybern I. B. (1965): The life cycle of *Ciona intestinallis* (L.) typica in relation to the environmental temperature. Oikos 16: 109-131.
27. Eckman, J. E. (1983): Hydrodynamic processes affecting benthic recruitment. Limnol Oceanogr., 28:241-257.
28. Gautier, P.; Olgun, K.; Uzum, N. and Miaud, C. (2006): Gregarious behavior in a salamander: attraction to conspecific chemical cues in burrow choice. Behav. Ecol. Sociobiol., 59:836-841.
29. Georges D. and Dubois M. P. (1984): Methionine-enkephalin-like immunoreactivity in the nervous ganglion and the ovary of a protochordate, *Ciona intestinalis*. Cell Tissue Res., 236:165-170.
30. Gilbert, S. F. and Raunio, A. M.(1997): Embryology: Constructing the Organism. Sinauer Associates, Inc.publishers Sunderland, MA 01375 USA. With illustrations by Haver N. J. Dedicated to Berrill, N. J. (1903-1996) and Samers M. E., powerful inducers who never lost focus on the whole embryo ISBN 0-87893-237-2.
31. Goodbody, I. and Fisher, E. (1974): The biology of *Ascidia nigra* (Savigny). IV. seasonal and spatial patterns of embryonic development and hatching success. J. Biol. Bull. 146 : 206-216
32. Goodbody, I. and Gibson , J. (1974): The biology of *Ascidia nigra* (Savigny).V. Survival in populAtions settled at different times of the year. J. Biol. Bull., 140 : 217-237.

© 2016 Global Journals Inc. (US)

33. Grave C. (1944): The larva of *Styela* (*Cynthia*) *partita* : Structure, Activities and duration of Life. J. Morph., 75:173-188.
34. Hadfield, M. G. and Paul, V. J. (2001): Natural chemical cues for settlement and metamorphosis of marine-invertebrate larvae. In: McClintock JB, Baker BJ (eds) Marine Chemical Ecology. CRC Press, Boca Raton, Florida, p 431-461.
35. Havenhand, J. N. and Svane, I. (1991): Roles of hydrodynamics and larval behavior in determining spatial aggregation in the tunicate *Ciona intestinalis*. Mar. Ecol. Prog .Ser., 68:271-276.
36. Hecht M. K. , Steere W. C. and Wallace B. (1977): Evolutionary Biology. volume 10-Plenum press, New York. A Division of Plenum Publishing Corporation 227 West 17th Street, New York, N. Y. 10011, printed in USA.
37. Hinz, H. L. and Schwarzlaender, M. (2004): Comparing invasive plants from their native and exotic range: What can we learn for biological control? Weed Technol., 18:1533- 1541.
38. Hofmann D.K., Boletzky S., Fleck J., Schwammberger K. H. and Rudschewski(1999): Protokolle über Untersuchungsreihen zur Entwicklungsgeschichte und Entwicklungsphysiologie ausgewählter mariner Invertebraten. Ruhr - Universität Bochum.
39. Hofmann, D.K.; Michael, M. I.; Khalil, S.H.; El-Bawab, F.M. and Saad G.A.(2008): Larval metamorphosis in Ascidiella aspersa (Müller, 1776) and Phallusia mammilata (Cuvier, 1815) Urochordata, Ascidiacea - An experimental study including an immunocytochemical approach. Proc. 5th Int.Con. Biol.(Zool5:235-2485:235.
40. Holmberg K. (1984): A transmission electron microscopic investigation of the sensory vesicle in the brain of O*ikopleura dioica* (appendicularia). Zoomorph., 104:298-303.
41. Howes. S.; Herbinger, C. M.;, Darnell, P. and Vercaemer, B. (2007): Spatial and temporal patterns of recruitment of the tunicate *Ciona intestinalis* on a mussel farm in Nova Scotia, Canada. J. Exp. Mar. Bol. Ecol., 342:85-92.
42. Jacobs, M. W.; Degnan, B. M.; Bishop, J. D. and Strathmann, R. R. (2008): Early activation of adult organ differentiation during delay of metamorphosis in solitary ascidians, and consequences for juvenile growth. Invertebr. Biol., 127:217-236.
43. Jiang, D.; Tresser, J. W.; Horie, T.; Tsuda, M. and Smith, W. C. (2005): Pigmentation in the sensory organs of the ascidian larva is essential for normal behavior. J Exp Biol 208:433-438.
44. Kasper, M. L.; Reeson, A. F. and Austin, A. D. (2008): Colony characteristics of *Vespula germanica* (F.) (Hymenoptera, Vespidae) in a Mediterranean climate (southern Australia). Aus. J. Entomol., 47:265-274.
45. Katz M. J. (1983): Comprative anatomy of the tunicate tadpole *Ciona intestinalis*. Biol. Bull., 164:1-27.
46. Keough, M. J. and Raimondi, P. T. (1995): Responses of settling invertebrate larvae to bioorganic films: effects of different types of films. J. Exp. Mar. Biol. Ecol., 185:235-253.
47. Knoke, D. and Burke, P. J. (1991): Log-linear Models. Quantitative Applications in the Social Sciences. Sage Publishers, Newbury Park, CA
48. Koh, E. G. and Sweatman, H. (2000): Chemical warfare among scleractinians: bioactive natural products from *Tubastraea faulkneri* Wells kill larvae of potential competitors. J. Exp. Mar. Biol. Ecol., 251:141-160.
49. Kriegel (1996): Immunocytochemische Versuche zur Darstellung von Nervenelementen in Entwicklungsstadien von *Cassiopea spp*.(Cnidaria: Scyphozoa). Schriftliche Hausarbeit im Rahmen der ersten Staatsprüfung Für das Lehramt für die Sekundarstufe II Dem Staatlichen Prüfungsamt Dortmund Themensteller : Prof. Dr. D. K. Hofmann.
50. Kott, P. (1985): The Australian Ascidiacea, Part 1. Phlebobranchia and Stolidobranchia. Mem. Qld. Mus., 23:1-438.
51. Kriegel (1996): Immunocytochemische Versuche zur Darstellung von Nervenelementen in Entwicklungsstadien von *Cassiopea spp*.(Cnidaria : Scyphozoa). Schriftliche Hausarbeit im Rahmen der ersten Staatsprüfung für das Lehramt für die Sekundarstufe II Dem Staatlichen Prüfungsamt Dortmund Themensteller : Prof. Dr. D. K.Hofmann Fachbereich Entwicklungsbiologie.
52. Lambert, C. C. and Lambert, G. (1998). Non-indigenous ascidians in southern California harbors and marinas. Mar. Biol., 130: 675-688.
53. Lambert, G. (2001). A global overview of ascidian introductions and their possible impact on the endemic fauna. In: Sawada, H., Yokosawa, H., Lambert, C.C. (Eds.), The Biology of Ascidians. Springer-Verlag, Tokyo.
54. Lambert, C. C. and Lambert, G. (2003): Persistence and differential distribution of nonindigenous ascidians in harbors of the Southern California Bight. Mar. Ecol. Prog. Ser., 259:145-161.
55. Lambert, G. (2005): Ecology and natural history of the protochordates. Can. J. Zool., 83:34- 50.
56. Lambert, G. (2007): Invasive sea squirts: A growing global problem. J. Exp. Mar. Biol. Ecol., 342:3-4.
57. Lübbering, B. (1994): Morphogenese et cytochimie de la tunique larvaire et juvenile des ascidies *Ascidiella aspersa* (Ascidiacea, Phlebobranchiata) et Halocynthia papillosa (Ascidiacea, Stolidobranchiata). Pour i'obtention du grade de Docteur en Sciences Faculte des Sciences, Universite de Liege.

© 2016 Global Journals Inc. (US)

58. Mackie, G. O.; Paul, D. M.; Singia, C. M.; Sleigh, M, A. and Williams, D.E.(1974): Branchial innervation and ciliary control in the ascidian *Corella.* Pro. Roy. Soc. Lond. B. 187 : 1-35.
59. Mancuso V. (1974): Formation of the ultrastructural components of *Ciona intestinalis* tadpole test by the animal embryo. Experentia, 30: 1078.
60. Marshall, D. J.; Styan, C. A. and Keough, M. J. (2000): Intraspecific covariation between egg and body size affects fertilization kinetics of free-spawning marine invertebrates. Mar. Ecol. Prog. Ser., 195:305-309.
61. Mastrototaro, F.; D'onghia, G. and Tursi, A. (2008): Spatial and seasonal distribution of ascidians in a semi-enclosed basin of the Mediterranean Sea. J. Mar. Biol. Ass., U K 88:1053-1061.
62. McHenry, M. J. and Patek, S. N. (2004): The evolution of larval morphology and swimming performance in ascidians. Evolution, 58:1209-1224.
63. Millar R. H. (1952): The annual growth and reproductive cycle in four ascidians. J. Marine Biol. Ass., 31: 41-61.
64. Millar R. H. (1971): The Biology of Ascidians. Adv. mar. Biol., 9: 1-100.
65. Miller, R. J. and Etter, R. J. (2008): Shading facilitates sessile invertebrate dominance in the rocky subtidal Gulf of Maine. Ecology. 89:452-462.
66. Monniot, C.; Monniot, F.; Griffiths, C. L. and Schleyer, M. (2001): South African ascidians. Ann-S- Afr- Mus-. 108:1-141.
67. Niermann-Kerkenberg(1989) : Entstehung, Struktur und Funktion der Zellulären Eihüllen von *Ascidella aspersa* (Tunicata) während der oogenese undEmbriologie. Zur Erlangung des Grades eines Doktors der Naturwissenschaften der Fakulität für Biologie an der Ruhr –Universität Bochum.
68. Niermann-Kergenberg, E. and Hofmann, D. K. (1989): Fertilization and normal development in *Ascidiella aspersa* (Tunicata) studied with Nomarski-optics. Helgol. Mar. Sci., 43:245-258.
69. Ohtsuki, H. (1990): Statocyte and ocellar pigment cell in embryos and larvae of the ascidian, *Styela plicata* (Lesueur). Dev. Growth Differ., 32:85-90.
70. Olsson R., Holmberg K. and Lilliemarck Y.(1990): Fine structure of the brain nerves of *Oikopleura dioica* (Urochordata-Appendicularia). Zoomorph., 110:1-7.
71. Quinn, G. P. and Keough, M. J. (2002): Experimental design and data analysis for biologists. Cambridge University Press, Cambridge, U. K.
72. Ramsay, A.; Davidson, J.; Landry, T. and Arsenault, G. (2008): Process of invasiveness among exotic tunicates in Prince Edward Island, Canada. Biol. Invasions., 10:1311-1316.
73. Rinkevich, B. and Weissman, I. L. (1987): The fate of *Botryllus* (Ascidiacea) larvae cosettled with parental colonies beneficial or deleterious cosequences ? Biol. Bull., 173(3): 474.
74. Rius, M. and McQuaid, C. D. (2009): Facilitation and competition between invasive and indigenous mussels over a gradient of physical stress. Basic Appl. Ecol., 10:607- 613.
75. Rius, M.; Pineda, M. C. and Turon, X. (2009a): Population dynamics and life cycle of the introduced ascidian *Microcosmus squamiger* in the Mediterranean Sea. Biol. Invasions, 11:2181-2194.
76. Rius, M.; Turon, X. and Marshall, D. J. (2009b): Non-lethal effects of an invasive species in the marine environment - the importance of early life-history stages. Oecologia, 159:873-882.
77. Robinson, T. B.; Griffiths, C. L.; McQuaid, C. D. and Rius, M. (2005): Marine alien species of South Africa - status and impacts. Afr. J. Mar. Sci., 27:297-306.
78. Rosati F. and De Santis R. (1978): Studies on fertilization in the ascidians.1. Self-sterility and specific recognition between gameters of *Ciona intestinalis*. Exp. Cell Res., 112:111-119.
79. Ross, C. A. and Auge, H. (2008): Invasive *Mahonia* plants outgrow their native relatives. Plant Ecol., 199:21-31.
80. Saad, G. A., Hamed, S. S. ; Radwan, Kh. H. and Radwan, E. H. (2010): Screening of genomic DNA and analysis of heavy metals to identify mutations in the genes of Ciona intestinalis (Linnaeus, 1767) collected from the Mediterranean Sea - Alexandria, Egypt. Scientific Research and Essays,, 6(23), 4984-5003. http://www.academicjournals.org/SRE
81. Saad, G. A. (2002): Comparative studies of the nervous and reproductive systems of some species of urochordates with emphasis of the role of the nervous system on Alexandria University reproduction and larval metamorphosis. Ph.D. Thesis, Faculty of Science.
82. Satoh, N.(1994): Developmental Biology of Ascidians Kyoto University Published by the Press Syndicate of the University of Cambridge. Printed in the united states of America. ISBN 0-521-35221-5.
83. Stachowicz, J. J.; Whitlatch, R.B.; Osman, R.W. (1999). Species diversity and invasion resistance in a marine ecosystem. Sci., 286 (5444): 1577-1579.
84. Stachowicz, J.J. ; Terwin, J. R. ; Whitlatch, R.B. and Osman, R.W. (2002). Linking climate change and biological invasions: ocean warming facilitates nonindigenous species invasions. Proc. Natl. Acad. Sci., 99 (24): 15497–15500.
85. Stern C.D. and Holland P.W.H. (1993): Essential developmental biology. A practical approach. The practical approach series editors: Rickwood D. and Hames B.D. Printed in Great Britain by Information Press Ltd, Eynsham, Oxford.
86. Svane, I.; Havenhand, J. N. and Jorgensen, A. J. (1987): Effects of tissue extract of adults on

© 2016 Global Journals Inc. (US)

metamorphosis in *Ascidia mentula* O. F. Muller and *Ascidiella scabra* (O. F. Muller). J. Exp. Mar. Biol. Ecol.,110:171-181.

87. Svane, I. and Young, C. M. (1989): The ecology and behavior of ascidian larvae. Oceanogr. Mar. Biol. Annu. Rev., 27:45-90.
88. Svane, I. and Young, C. M. (1991): Sensory structures in tadpole larvae of the ascidians *Microcosmus exaspertus* Heller and *Herdmania momus* (Savigny). Acta Zool., 72:129-135.
89. Tannenbaum A. S. and Rosenbluth J. (1972): Myoneural junctions in larval ascidian tail. Experientia, 28(2): 1210-1212.
90. Torrence S. A. and Cloney R. A. (1982): Nervous System of ascidian larvae: Caudal Primary sensory Neurons. Zoomorph., 99: 103-115.
91. Torrence , S. A . and Cloney , R. A . (1983): Ascidian larval nervous system, primary sensory neurons in adhesive papillae. Zoomorph., 102: 111-123.
92. Underwood, A. J. and Keough, M. J. (2001): Supply-side ecology: The nature and consequences of variations in recruitment of intertidal organisms. In: Bertness MD, Gaines SD, Hay ME (eds) Marine community ecology. Sinauer Assoc., Sunderland, Pp 183-200.
93. Valiela, I.; Collins, G.; Kremer, J.; Lajtha, K.; Geist, M.; Seely, B.; Brawley, J. and Sham, C.H. (1997). Nitrogen loading from coastal watersheds to receiving estuaries: new method and application. Ecol. Appl., 7 (2): 358-380.
94. Westheide W. and Rieger R. (1996): Spezielle Zoologie.Erster Teil: Einzeller und Wirbellose Tiere. Gustav Fischer Verlag- Stuttgart – Jena - New York.
95. Whiteley, J. and Bendell-Young, L. (2007): Ecological implications of intertidal mariculture: observed differences in bivalve community structure between farm and reference sites. J. Appl. Ecol., 44:495-505.
96. Wolpert, L. M. (1999): Why alien invaders succeed: Support for the escape-from-enemy hypothesis. Am. Nat., 160:705-711.
97. Woollacott R. M. (1977): Spermatozoa of *Ciona intestinalis* and analysis of ascidian fertilization. J. Morph., 152:77-88.
98. Yamaguchi M. (1975): Growth and reproductive cycles of the marine fouling ascidians *Ciona intestinalis, Styela placata, Botrylloides violaceus* and *Leptoclinum mitsukurii* at Aburatsubo-Moeroiso Inlet (Central Japan). Marine Biol., 29,253-259.
99. Young, C. M. and Braithwaite, L. F. (1980): Orientation and current-induced flow in the stalked ascidian *Styela montereyensis*. Biol. Bull., 159:428-440.
100. Young, C. M. and Chia, F. S. (1985): An experimental test of shadow response function in ascidian tadpoles. J. Exp. Mar. Biol. Ecol., 85:165-175.
101. Young C. M., Gowan R. F., Dalby JR J., Pennachettf C. A. and Gagllardf D. (1988): Distributional consequences of adhesive eggs and anural development in the ascidian *Molgula pacifica* (Huntsman,1912). Biol. Bull., 174:39-46.

© 2016 Global Journals Inc. (US)

Histograms

Histogram 1. Adult distribution in the field, indicated as the mean density of individuals, and mean light intensity (μ mol m-2 s-1) in relation to surface orientation. Error bars denote + 1 SE. Note differences in scales of y-axes

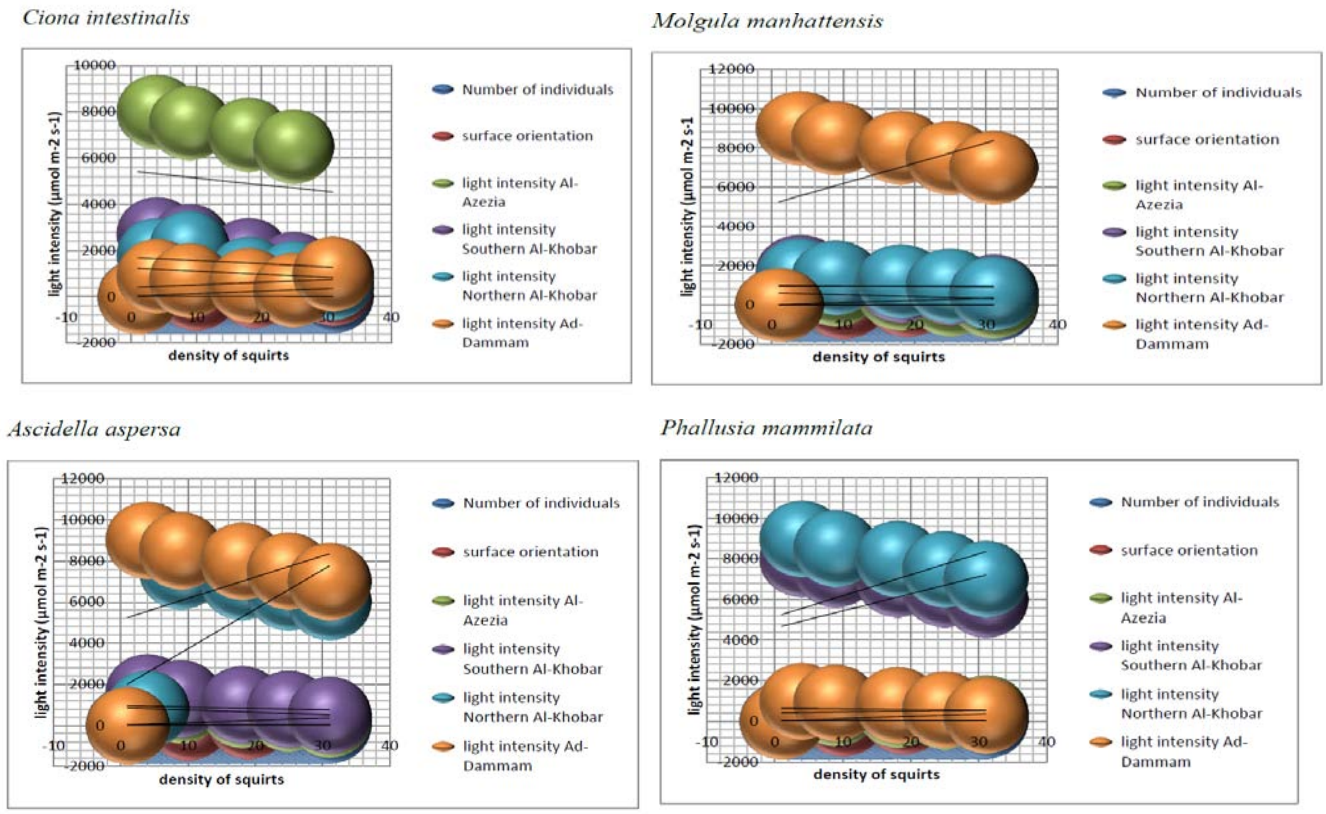
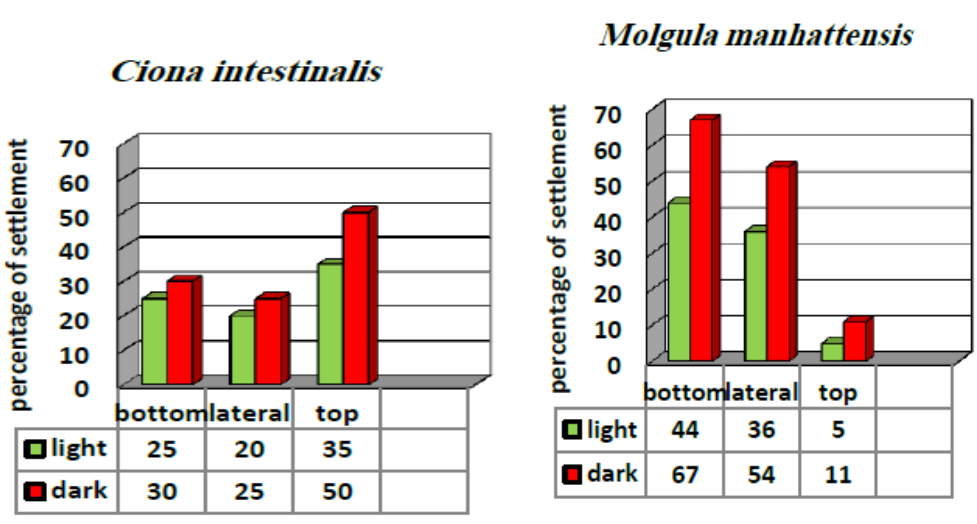


Fig. 1 : Phase contrast photomicrograph of a whole mount of a hatching larval stage of Ciona intestinalis (beginning of differentiation of the trunk and tail regions)

Histogram 2. Mean percentage settlement in relation to orientation (bottom, lateral or top) and treatment (light - green bars, dark - red bars) in the 1st experiment, in which larvae were held either in the dark or in the light. Error bars denote + 1 SE. Note differences in scales of y-axes



© 2016 Global Journals Inc. (US)

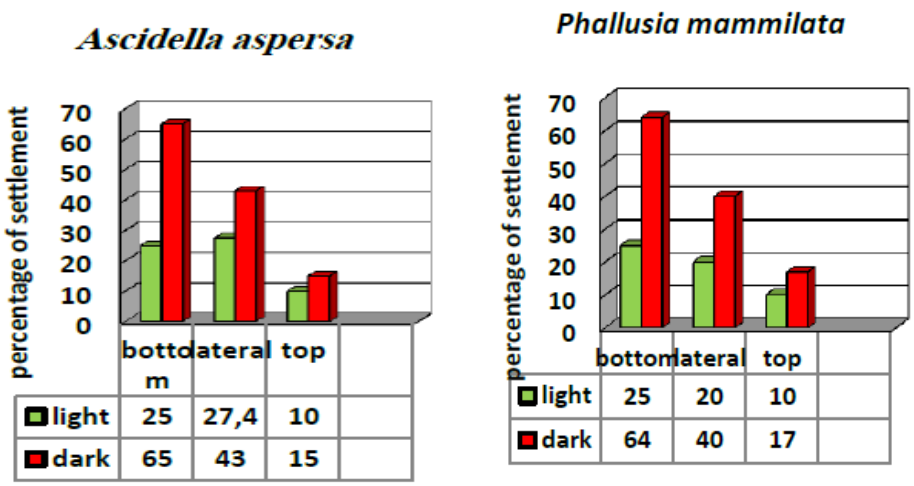


Fig. 2 : Phase contrast photomicrograph of a whole mount of a larval stage of Ciona intestinalis (Early tail resorption is indicated by an arrow)

Histogram 3. Mean percentage settlement in relation to orientation (bottom, lateral and top) and treatment (light - green bars, dark - red bars) in the 2nd experiment, in which larvae had the choice of settling in light or dark positions of the same wells. Error bars denote + 1 SE. Note differences in the scales of y-axes.

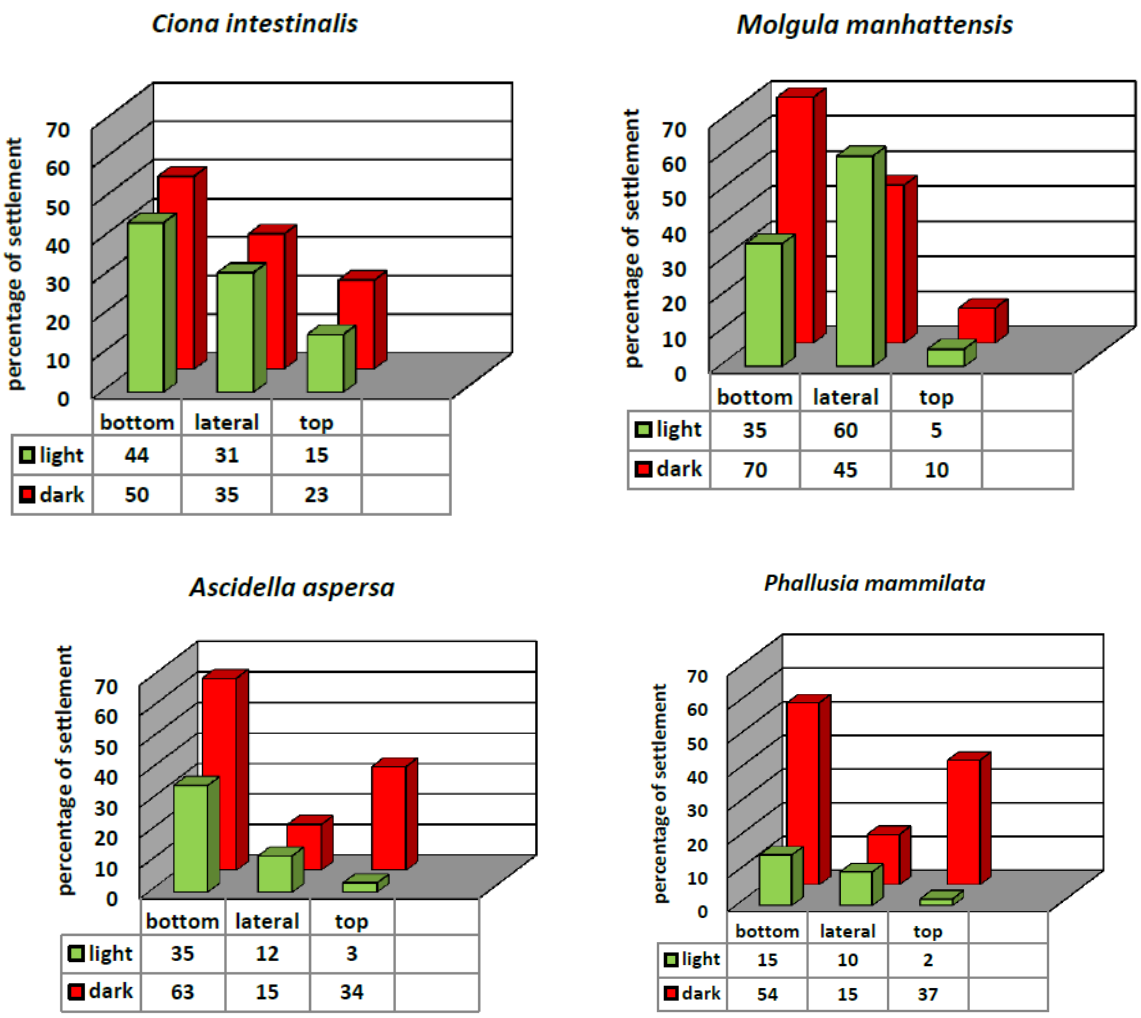


Fig. 3 : Phase contrast photomicrograph of a whole mount of a late metamorphosed larval stage of Ciona intestinalis

© 2016 Global Journals Inc. (US)

Histogram 4. Mean percentage settlement with respect to orientation (bottom, lateral and top) and treatment (control - blue bars, mantle tissues extract - brown bars) in the 3rd experiment, in which larvae were held in wells either with or without adult extract. Error bars denote + 1 SE. Note differences in the scales of y-axes.

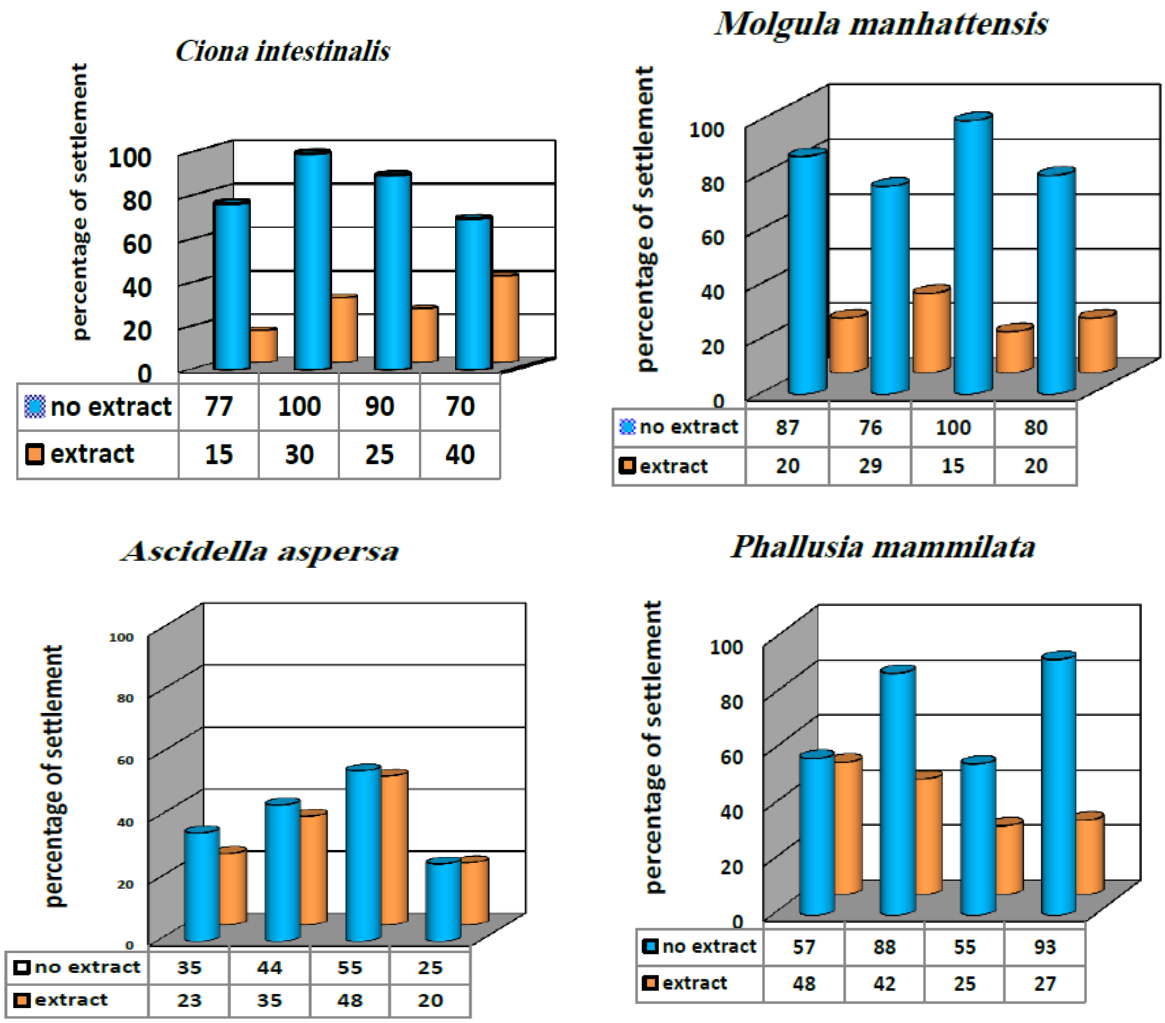
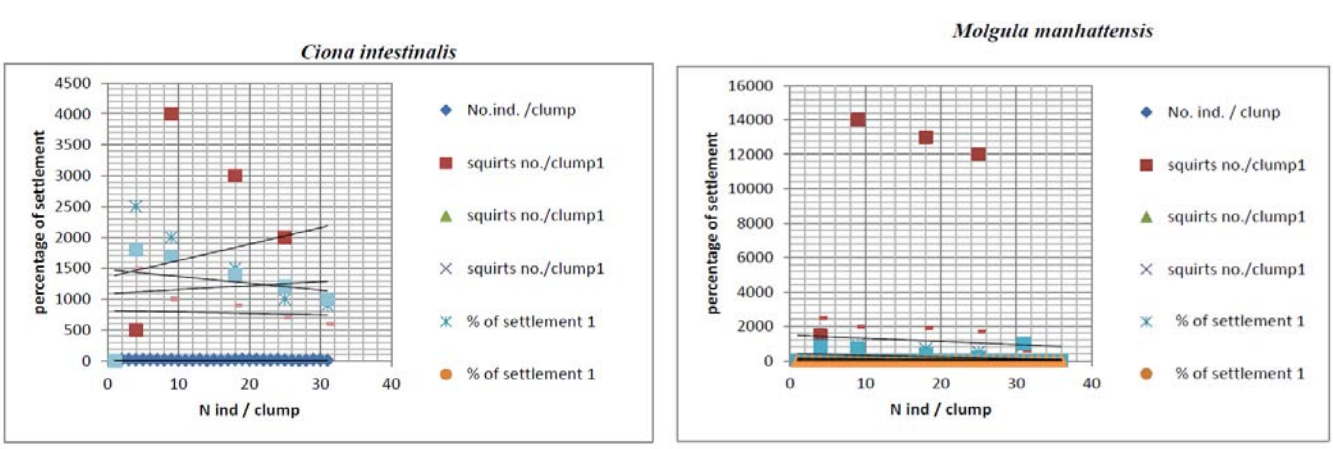
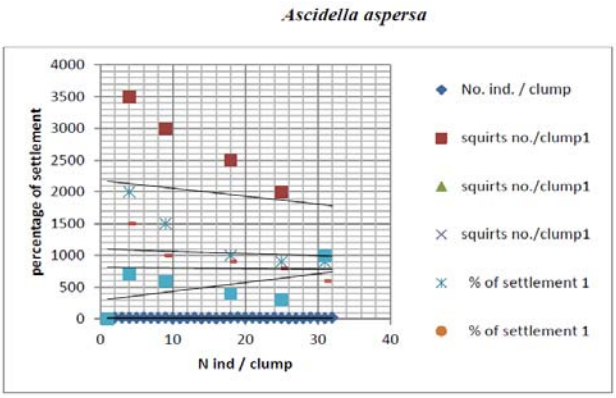


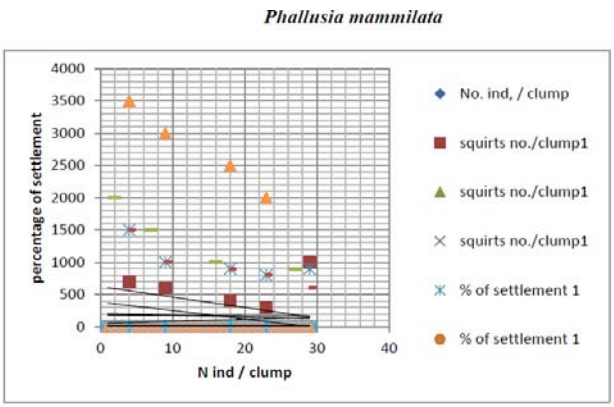
Fig. 4 : Photomicrograph of a transverse section of Phallusia mammilata showing the gonad. The squirt was collected during summer

Histogram 5. Mean percentage of settling larvae per clump from all the experiments, in relation to orientation. Error bars denote +1 SE. Note differences in scales of y-axes



© 2016 Global Journals Inc. (US)

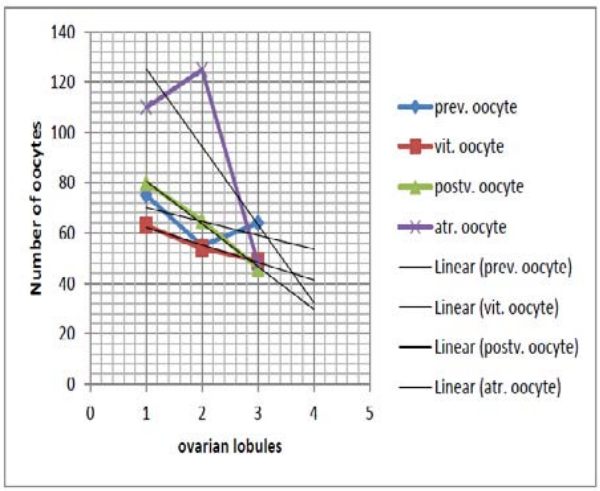




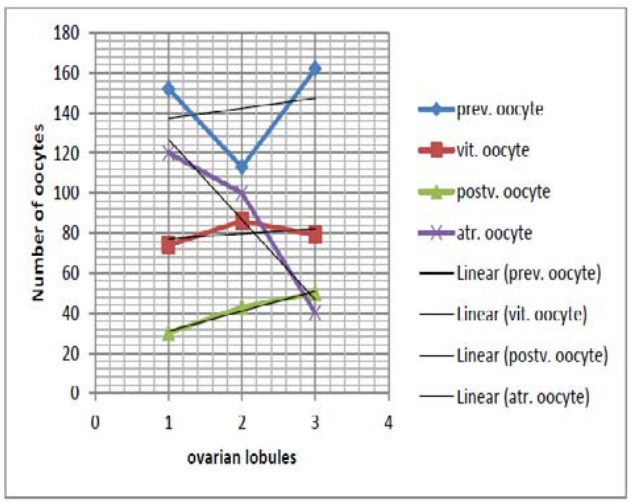
Figs. 5-6 : Photomicrograph of a transverse section of Phallusia mammilata ovary showing germinal epithelium and previtellogenic oocytes. The squirt was collected during spring

Histogram 6. Mean percentage of oocyte developmental stages of *Phallusia mammilata* along the four seasons of the year. Error bars denote +1 SE. Note differences in scales of y-axes

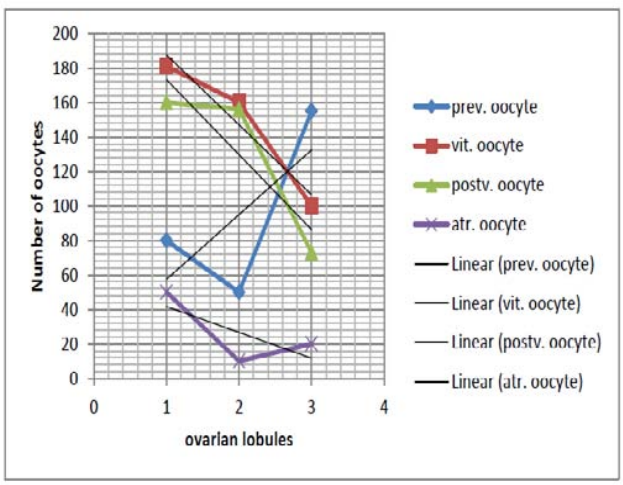
During spring



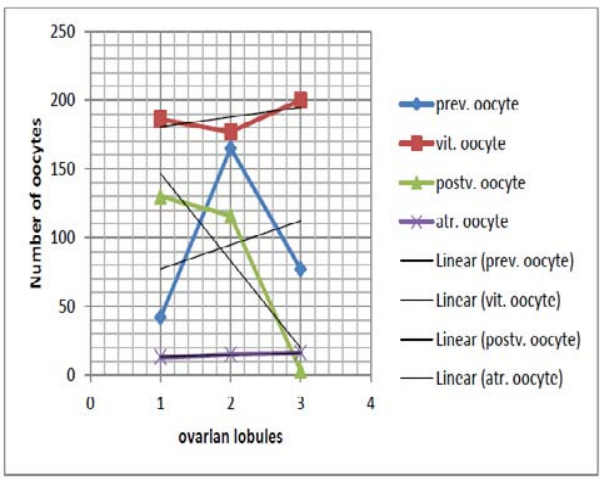
During summer



During autumn



During winter



© 2016 Global Journals Inc. (US)

Tables

Table 1 : Comparison of squirts density in the field, Note: One-way analysis of variance and Bartlett's test for equal variances were applied

Statistical analysis		*Ciona intestinalis*	*Molgula manhattensis*	*Ascidella aspersa*	*Phallusia mammilata*
One-way analysis of variance	P value	0,0023	P<0.0001	0,0006	P<0.0001
	Are means signif. different? (P < 0.05)	Yes	Yes	Yes	Yes
	Number of groups	4	4	4	4
	F	6,847	14,07	9,025	13,16
	R squared	0,5067	0,6896	0,5751	0,6638
Bartlett's test for equal variances	P value	P<0.0001	P<0.0001	P<0,0001	P<0.0001
	Do the variances differ signif. (P < 0.05)	Yes	Yes	Yes	Yes

Table 2 : Tukey's Multiple Comparison Test explaining mean percentage of settlement in relation to squirts orientation towards light direction

Ciona intestinalis				
Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
bottom vs lateral	6,5	1,019	P > 0.05	-20.29 to 33.29
bottom vs top	26,13	4,094	P > 0.05	-0.6672 to 52.92
bottom vs bottom	25,75	4,036	P > 0.05	-1.042 to 52.54
lateral vs top	19,63	3,076	P > 0.05	-7.167 to 46.42
lateral vs bottom	19,25	3,017	P > 0.05	-7.542 to 46.04
top vs bottom	-0,375	0,05877	P > 0.05	-27.17 to 26.42
Molgula manhattensis				
Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
bottom vs lateral	35	5,286	P < 0.05	5.015 to 64.99
bottom vs top	44,67	6,747	P < 0.01	14.68 to 74.65
bottom vs bottom	-10	1,51	P > 0.05	-39.99 to 19.99
lateral vs top	9,667	1,46	P > 0.05	-20.32 to 39.65
lateral vs bottom	-45	6,797	P < 0.01	-74.99 to -15.01
top vs bottom	-54,67	8,257	P < 0.01	-84.65 to -24.68
Ascidella aspersa				
Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
bottom vs lateral	15,63	1,391	P > 0.05	-31.54 to 62.79
bottom vs top	25,25	2,248	P > 0.05	-21.91 to 72.41
bottom vs bottom	8,375	0,7456	P > 0.05	-38.79 to 55.54
lateral vs top	9,625	0,8569	P > 0.05	-37.54 to 56.79
lateral vs bottom	-7,25	0,6455	P > 0.05	-54.41 to 39.91
top vs bottom	-16,88	1,502	P > 0.05	-64.04 to 30.29
Phallusia mammilata				
Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
bottom vs lateral	37,33	3,035	P > 0.05	-18.38 to 93.04
bottom vs top	51,33	4,173	P > 0.05	-4.376 to 107.0
bottom vs bottom	6,667	0,542	P > 0.05	-49.04 to 62.38
lateral vs top	14	1,138	P > 0.05	-41.71 to 69.71
lateral vs bottom	-30,67	2,493	P > 0.05	-86.38 to 25.04
top vs bottom	-44,67	3,631	P > 0.05	-100.4 to 11.04

© 2016 Global Journals Inc. (US)

Table 3 : Tukey's Multiple Comparison Test explaining mean percentage of settlement in which larvae had the choice of settling in light or dark positions in the same wells of the tissue culture plates of the experiment

Ciona intestinalis				
Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
dark vs light	8	2,508	P > 0.05	-6.446 to 22.45
dark vs dark	12,17	3,814	P > 0.05	-2.279 to 26.61
dark vs light	14,5	4,546	P < 0.05	0.05427 to 28.95
light vs dark	4,167	1,306	P > 0.05	-10.28 to 18.61
light vs light	6,5	2,038	P > 0.05	-7.946 to 20.95
dark vs light	2,333	0,7315	P > 0.05	-12.11 to 16.78
Molgula manhattensis				
Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
dark vs light	20,33	6,686	P < 0.01	6.559 to 34.11
dark vs dark	10,33	3,398	P > 0.05	-3.441 to 24.11
dark vs light	23,17	7,617	P < 0.01	9.392 to 36.94
light vs dark	-10	3,288	P > 0.05	-23.77 to 3.774
light vs light	2,833	0,9316	P > 0.05	-10.94 to 16.61
dark vs light	12,83	4,22	P > 0.05	-0.9411 to 26.61
Ascidella aspersa				
Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
dark vs light	4,667	1,207	P > 0.05	-12.84 to 22.17
dark vs dark	-11,33	2,932	P > 0.05	-28.84 to 6.171
dark vs light	9,833	2,544	P > 0.05	-7.671 to 27.34
light vs dark	-16	4,14	P > 0.05	-33.50 to 1.504
light vs light	5,167	1,337	P > 0.05	-12.34 to 22.67
dark vs light	21,17	5,477	P < 0.05	3.663 to 38.67
Phallusia mammilata				
Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
dark vs light	7,333	1,775	P > 0.05	-11.38 to 26.05
dark vs dark	-14,33	3,469	P > 0.05	-33.05 to 4.382
dark vs light	7,833	1,896	P > 0.05	-10.88 to 26.55
light vs dark	-21,67	5,243	P < 0.05	-40.38 to -2.951
light vs light	0,5	0,121	P > 0.05	-18.22 to 19.22
dark vs light	22,17	5,364	P < 0.05	3.451 to 40.88

Table 4 : Tukey's Multiple Comparison Test explaining mean percentage of settlement in which larvae were held in wells either with or without adult mantle tissues extract

Ciona intestinalis				
Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
no extract vs extract	10,5	3,372	P > 0.05	-3.601 to 24.60
no extract vs no extract	-7,333	2,355	P > 0.05	-21.43 to 6.768
no extract vs extract	11	3,533	P > 0.05	-3.101 to 25.10
extract vs no extract	-17,83	5,728	P < 0.05	-31.93 to -3.732
extract vs extract	0,5	0,1606	P > 0.05	-13.60 to 14.60
no extract vs extract	18,33	5,888	P < 0.05	4.232 to 32.43
Molgula manhattensis				
Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
no extract vs extract	21,17	5,109	P < 0.05	2.402 to 39.93
no extract vs no extract	4,167	1,006	P > 0.05	-14.60 to 22.93
no extract vs extract	20,33	4,908	P < 0.05	1.568 to 39.10
extract vs no extract	-17	4,103	P > 0.05	-35.76 to 1.765
extract vs extract	-0,8333	0,2011	P > 0.05	-19.60 to 17.93
no extract vs extract	16,17	3,902	P > 0.05	-2.598 to 34.93
Ascidella aspersa				
Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
no extract vs extract	5,333	3,314	P > 0.05	-1.956 to 12.62
no extract vs no extract	-2	1,243	P > 0.05	-9.289 to 5.289

© 2016 Global Journals Inc. (US)

no extract vs extract	6	3,728	P > 0.05	-1.289 to 13.29
extract vs no extract	-7,333	4,556	P < 0.05	-14.62 to -0.04421
extract vs extract	0,6667	0,4142	P > 0.05	-6.622 to 7.956
no extract vs extract	8	4,971	P < 0.05	0.7109 to 15.29
Phallusia mammilata				
Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
no extract vs extract	5,333	3,314	P > 0.05	-1.956 to 12.62
no extract vs no extract	-2	1,243	P > 0.05	-9.289 to 5.289
no extract vs extract	6	3,728	P > 0.05	-1.289 to 13.29
extract vs no extract	-7,333	4,556	P < 0.05	-14.62 to -0.04421
extract vs extract	0,6667	0,4142	P > 0.05	-6.622 to 7.956
no extract vs extract	8	4,971	P < 0.05	0.7109 to 15.29

Table 5 : Tukey's Multiple Comparison Test explaining mean number of squirts per clump in the field and settling larvae of all experiments in relation to orientation

Ciona intestinalis				
Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
squirts no./clump1 vs % of settlement 1	433,3	1,085	P > 0.05	-1147 to 2014
squirts no./clump1 vs squirts no ./clump2	966,7	2,42	P > 0.05	-614.1 to 2547
squirts no./clump1 vs % of settlement 2	566,7	1,419	P > 0.05	-1014 to 2147
% of settlement 1 vs squirts no ./clump2	533,3	1,335	P > 0.05	-1047 to 2114
% of settlement 1 vs % of settlement 2	133,3	0,3339	P > 0.05	-1447 to 1714
squirts no ./clump2 vs % of settlement 2	-400	1,002	P > 0.05	-1981 to 1181
Molgula manhattensis				
Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
squirts no./clump1 vs % of settlement 1	6250	4,503	P < 0.05	757.0 to 11740
squirts no./clump1 vs squirts no ./clump2	5467	3,939	P > 0.05	-26.32 to 10960
squirts no./clump1 vs % of settlement 2	6400	4,612	P < 0.05	907.0 to 11890
% of settlement 1 vs squirts no ./clump2	-783,3	0,5644	P > 0.05	-6276 to 4710
% of settlement 1 vs % of settlement 2	150	0,1081	P > 0.05	-5343 to 5643
squirts no ./clump2 vs % of settlement 2	933,3	0,6725	P > 0.05	-4560 to 6426
Ascidella aspersa				
Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
squirts no./clump1 vs % of settlement 1	950	2,935	P > 0.05	-331.3 to 2231
squirts no./clump1 vs squirts no ./clump2	1200	3,707	P > 0.05	-81.27 to 2481
squirts no./clump1 vs % of settlement 2	1500	4,634	P < 0.05	218.7 to 2781
% of settlement 1 vs squirts no ./clump2	250	0,7723	P > 0.05	-1031 to 1531
% of settlement 1 vs % of settlement 2	550	1,699	P > 0.05	-731.3 to 1831
% of settlement 3 vs % of settlement 4	300	0,9267	P > 0.05	-981.3 to 1581
Phallusia mammilata				
Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
squirts no./clump1 vs % of settlement 1	-350	1,714	P > 0.05	-1149 to 449.5
squirts no./clump1 vs squirts no ./clump2	-509,1	2,836	P > 0.05	-1212 to 193.7
squirts no./clump1 vs % of settlement 2	-2250	9,856	P < 0.001	-3144 to -1356
% of settlement 1 vs squirts no ./clump2	-159,1	0,8863	P > 0.05	-861.9 to 543.7
% of settlement 1 vs % of settlement 2	-1900	8,322	P < 0.001	-2794 to -1006
squirts no ./clump2 vs % of settlement 2	-1741	8,43	P < 0.001	-2549 to -932.4

Table 6 : Tukey's Multiple Comparison Test explaining mean percentage of oocyte developmental stages of *Phallusia mammilata* along spring season

Table Analyzed				
Data Table-% of oocyte developmental stages along autumn season.				
One-way analysis of variance				
P value	0,2516			
P value summary	ns			
Are means signif. different? (P < 0.05)	No			
Number of groups	4			

© 2016 Global Journals Inc. (US)

F	1,66			
R squared	0,3837			
ANOVA Table	SS	df	MS	
Treatment (between columns)	2624	3	874,8	
Residual (within columns)	4215	8	526,8	
Total	6839	11		
Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
prev. oocyte vs vit. oocyte	9,333	0,7043	P > 0.05	-50.68 to 69.35
prev. oocyte vs postv. oocyte	1	0,07546	P > 0.05	-59.02 to 61.02
prev. oocyte vs atr. oocyte	-29,67	2,239	P > 0.05	-89.68 to 30.35
vit. oocyte vs postv. oocyte	-8,333	0,6288	P > 0.05	-68.35 to 51.68
vit. oocyte vs atr. oocyte	-39	2,943	P > 0.05	-99.02 to 21.02
postv. oocyte vs atr. oocyte	-30,67	2,314	P > 0.05	-90.68 to 29.35

Table 7 : Tukey's Multiple Comparison Test explaining mean percentage of oocyte developmental stages of *Phallusia mammilata* along summer season

Table Analyzed				
Data Table--% of oocyte developmental stages along summer season.				
One-way analysis of variance				
P value	0,0079			
P value summary	**			
Are means signif. different? (P < 0.05)	Yes			
Number of groups	4			
F	8,228			
R squared	0,7552			
ANOVA Table	SS	df	MS	
Treatment (between columns)	15690	3	5231	
Residual (within columns)	5086	8	635,8	
Total	20780	11		
Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
prev. oocyte vs vit. oocyte	62,67	4,305	P > 0.05	-3.264 to 128.6
prev. oocyte vs postv. oocyte	101,3	6,961	P < 0.01	35.40 to 167.3
prev. oocyte vs atr. oocyte	55,67	3,824	P > 0.05	-10.26 to 121.6
vit. oocyte vs postv. oocyte	38,67	2,656	P > 0.05	-27.26 to 104.6
vit. oocyte vs atr. oocyte	-7	0,4809	P > 0.05	-72.93 to 58.93
postv. oocyte vs atr. oocyte	-45,67	3,137	P > 0.05	-111.6 to 20.26

Table 8 : Tukey's Multiple Comparison Test explaining mean percentage of oocyte developmental stages of *Phallusia mammilata* along autumn season

Table Analyzed				
Data Table- % of oocyte developmental stages along autumn season.				
One-way analysis of variance				
P value	0,0394			
P value summary	*			
Are means signif. different? (P < 0.05)	Yes			
Number of groups	4			
F	4,506			

© 2016 Global Journals Inc. (US)

R squared	0,6282			
ANOVA Table	SS	df	MS	
Treatment (between columns)	25470	3	8491	
Residual (within columns)	15080	8	1884	
Total	40550	11		
Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
prev. oocyte vs vit. oocyte	-52	2,075	P > 0.05	-165.5 to 61.51
prev. oocyte vs postv. oocyte	-34,67	1,383	P > 0.05	-148.2 to 78.84
prev. oocyte vs atr. oocyte	68,33	2,726	P > 0.05	-45.18 to 181.8
vit. oocyte vs postv. oocyte	17,33	0,6916	P > 0.05	-96.18 to 130.8
vit. oocyte vs atr. oocyte	120,3	4,801	P < 0.05	6.824 to 233.8
postv. oocyte vs atr. oocyte	103	4,11	P > 0.05	-10.51 to 216.5

Table 9 : Tukey's Multiple Comparison Test explaining mean percentage of oocyte developmental stages of *Phallusia mammilata* along winter season

Table Analyzed				
Data Table-% of oocyte developmental stages along winter season.				
One-way analysis of variance				
P value	0,0139			
P value summary	*			
Are means signif. different? (P < 0.05)	Yes			
Number of groups	4			
F	6,747			
R squared	0,7167			
ANOVA Table	SS	df	MS	
Treatment (between columns)	45550	3	15180	
Residual (within columns)	18000	8	2251	
Total	63560	11		
Tukey's Multiple Comparison Test	Mean Diff.	q	P value	95% CI of diff
prev. oocyte vs vit. oocyte	-93	3,396	P > 0.05	-217.0 to 31.05
prev. oocyte vs postv. oocyte	11,67	0,426	P > 0.05	-112.4 to 135.7
prev. oocyte vs atr. oocyte	80	2,921	P > 0.05	-44.05 to 204.0
vit. oocyte vs postv. oocyte	104,7	3,821	P > 0.05	-19.38 to 228.7
vit. oocyte vs atr. oocyte	173	6,316	P < 0.01	48.95 to 297.0
postv. oocyte vs atr. oocyte	68,33	2,495	P > 0.05	-55.71 to 192.4

© 2016 Global Journals Inc. (US)

Legends

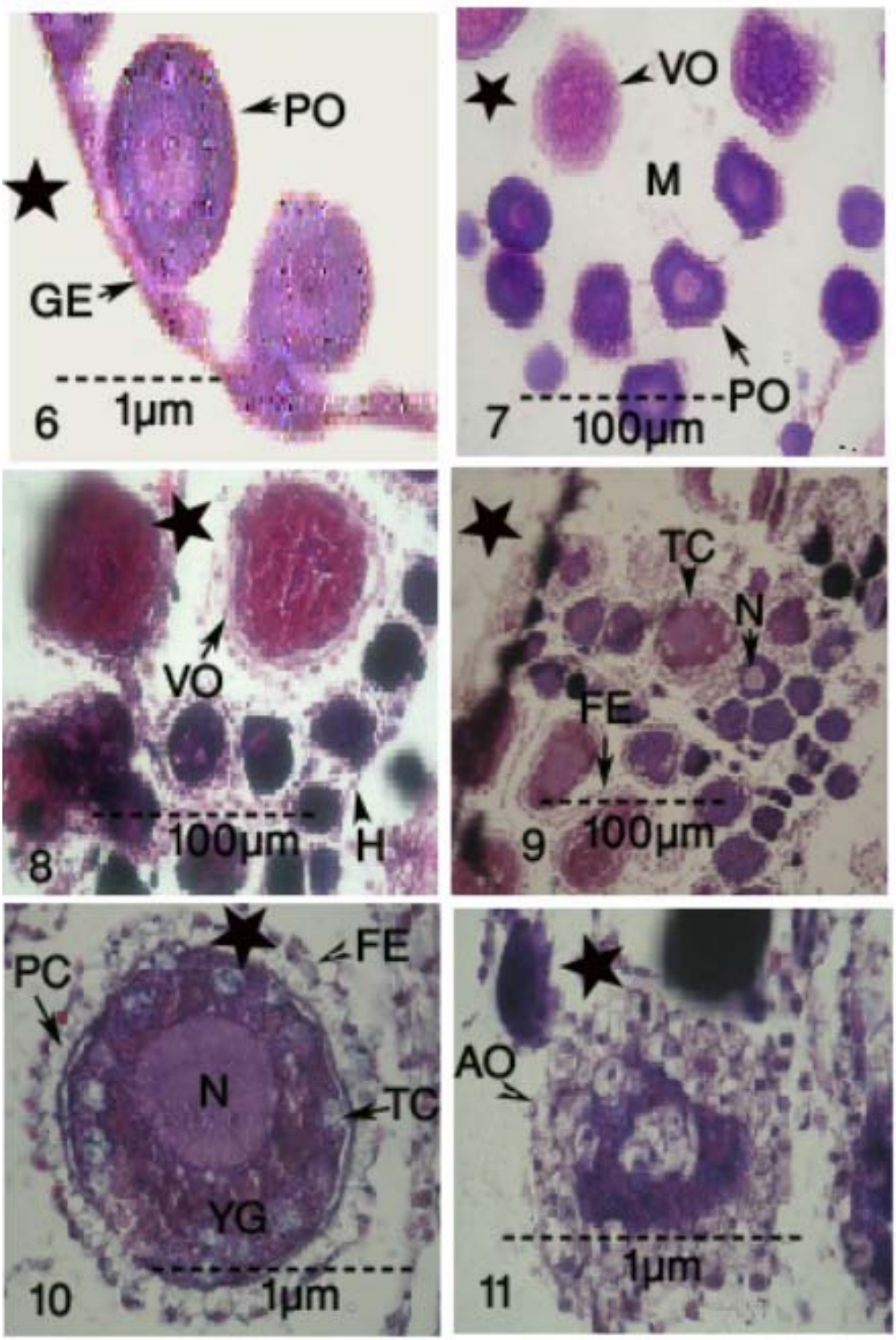
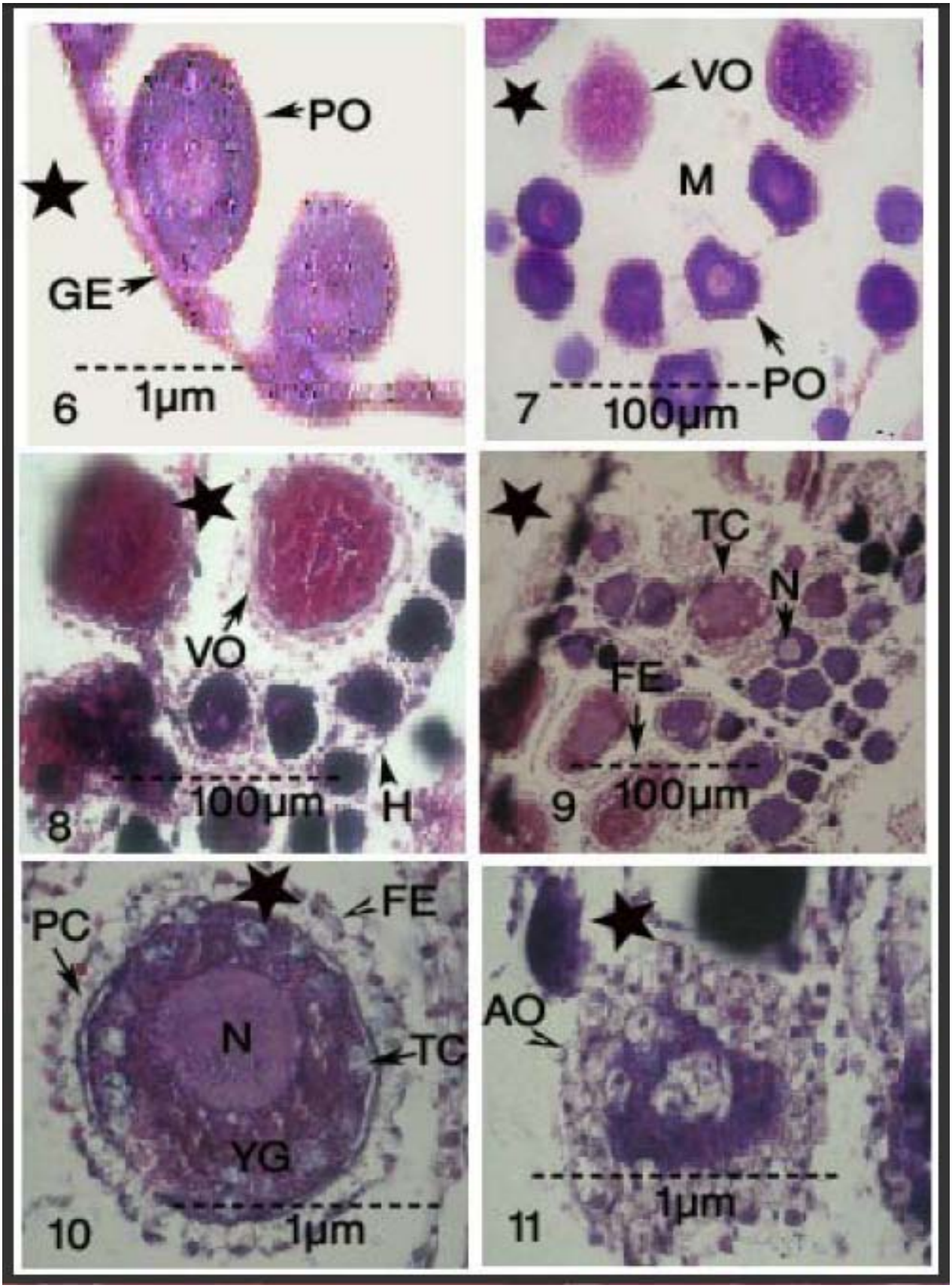


Fig. 7 : Photomicrograph of a transverse section of *Phallusia mammilata* ovary showing previtellogenic and early stage of vitellogenic oocytes. The squirt was collected during summer

© 2016 Global Journals Inc. (US)



Figs. 8-9 : Photomicrograph of a transverse section of *Phallusia mammilata* ovary showing vitellogenic oocytes. Note, follicular epithelium and test cells were developed. The squirt was collected during autumn.

© 2016 Global Journals Inc. (US)

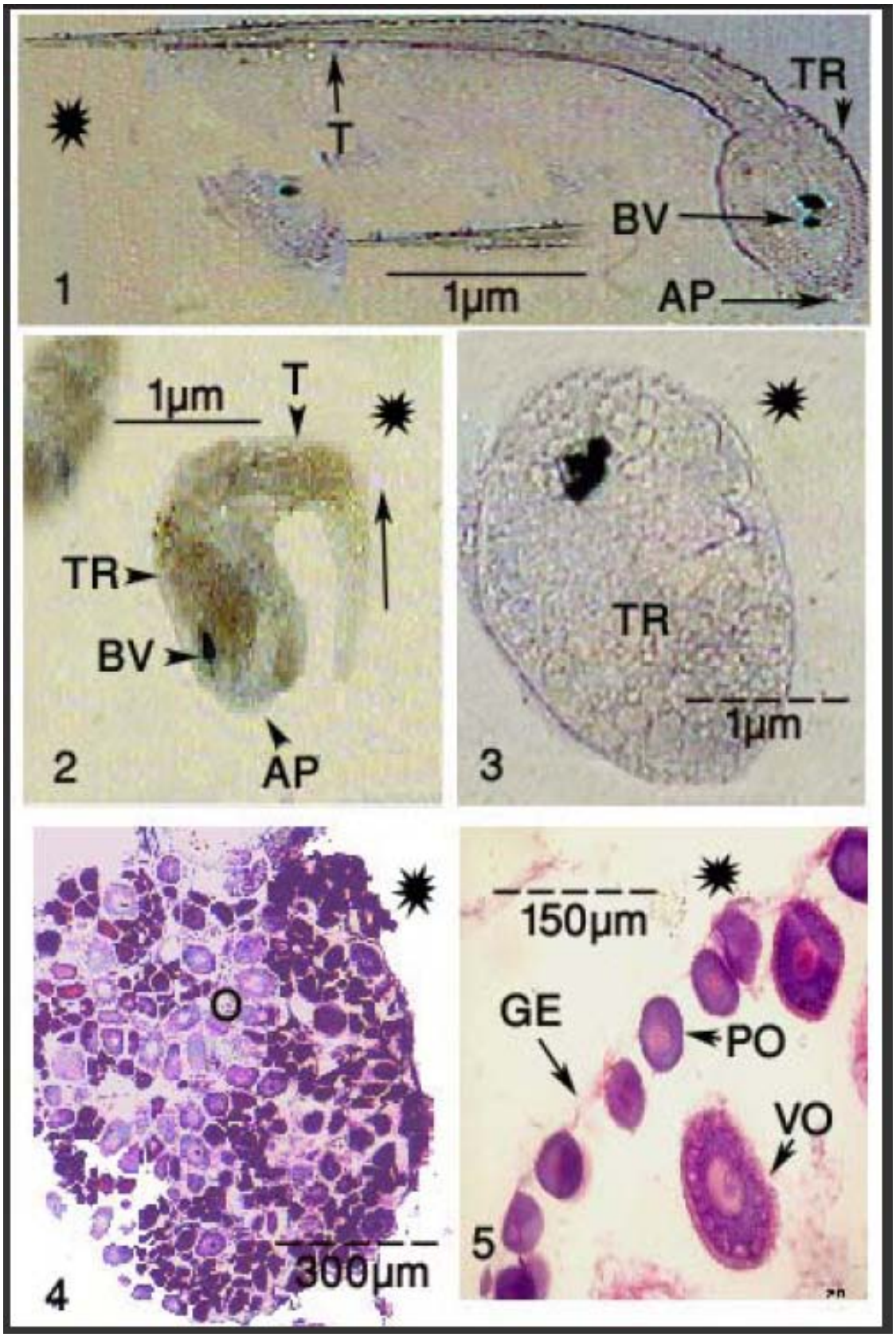


Fig. 10 : Photomicrograph of a transverse section of *Phallusia mammilata* ovary showing late stage of vitellogenic oocytes. Note, follicular epithelium, test cells and pervitelline space were developed. The squirt was collected during winter.

© 2016 Global Journals Inc. (US)

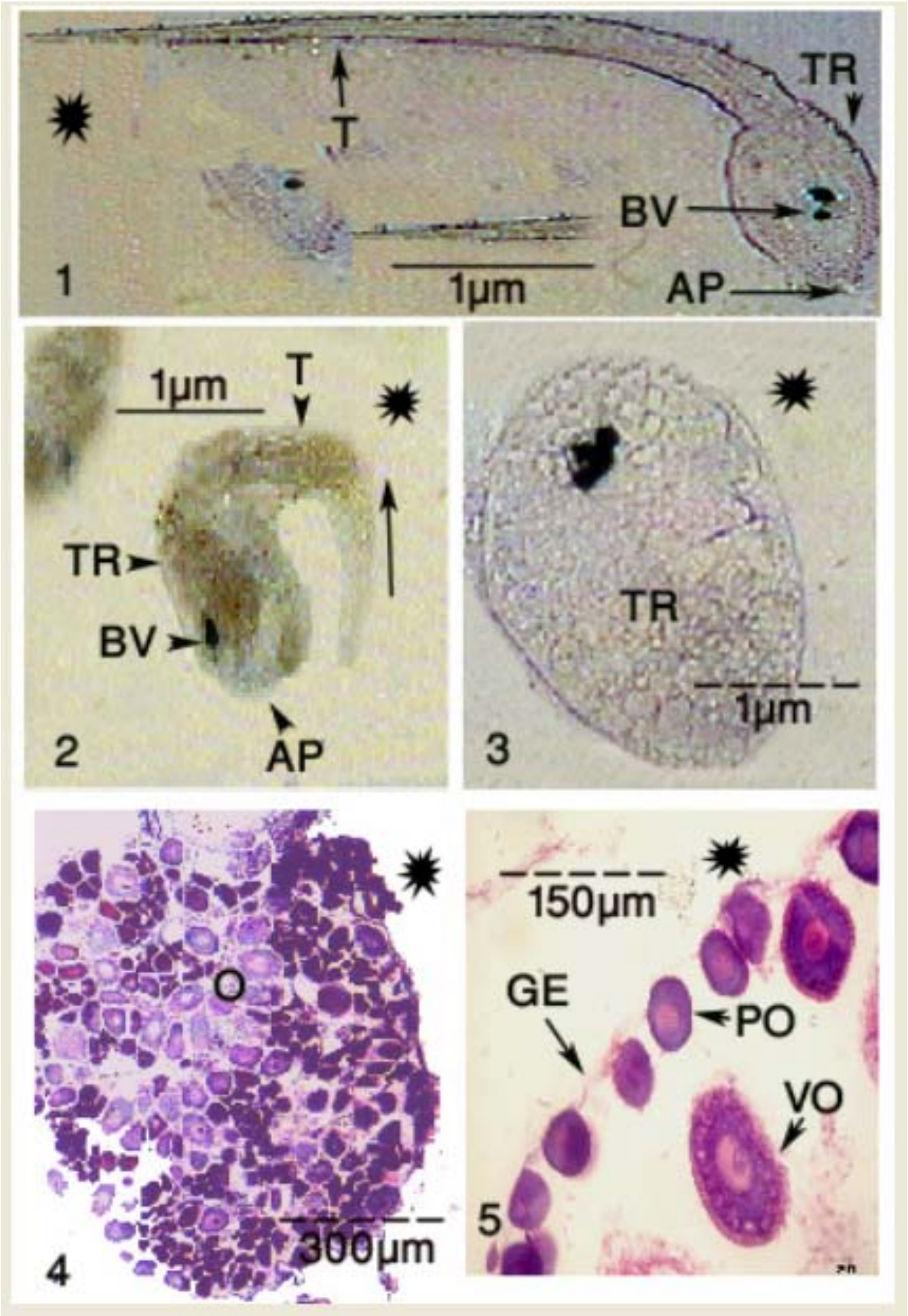


Fig. 11 : Photomicrograph of a transverse section of *Phallusia mammilata* ovary showing atretic oocytes. The squirt was collected during late winter.

© 2016 Global Journals Inc. (US)

Figs. 11 : Photomicrograph of a transverse section of *Phallusia mammilata* ovary showing atretic oocytes. The squirt was collected during late winter

Abbreviations

AO	Atretic Oocyte
AP	Adhesive Papillae
BV	Brain Vesicle
FE	Follicular Epithelium
HC	Haemocyte
O	Ovary
PO	Previtellogenic Oocyte
T	Tail
TR	Trunk
VO	Vitellogenic Oocyte
GE	Germinal Epithelium
M	Matrix
PS	Pervitelline Space
M	Martix
TC	Test Cell
N	Nucleus
YG	Yolk Granules

© 2016 Global Journals Inc. (US)

Global Journals Inc. (US) Guidelines Handbook 2016

www.GlobalJournals.org

FELLOWS

FELLOW OF ASSOCIATION OF RESEARCH SOCIETY IN SCIENCE (FARSS)

Global Journals Incorporate (USA) is accredited by Open Association of Research Society (OARS), U.S.A and in turn, awards "FARSS" title to individuals. The 'FARSS' title is accorded to a selected professional after the approval of the Editor-in-Chief/Editorial Board Members/Dean.

- The "FARSS" is a dignified title which is accorded to a person's name viz. Dr. John E. Hall, Ph.D., FARSS or William Walldroff, M.S., FARSS.

FARSS accrediting is an honor. It authenticates your research activities. After recognition as FARSB, you can add 'FARSS' title with your name as you use this recognition as additional suffix to your status. This will definitely enhance and add more value and repute to your name. You may use it on your professional Counseling Materials such as CV, Resume, and Visiting Card etc.

The following benefits can be availed by you only for next three years from the date of certification:

FARSS designated members are entitled to avail a 40% discount while publishing their research papers (of a single author) with Global Journals Incorporation (USA), if the same is accepted by Editorial Board/Peer Reviewers. If you are a main author or co-author in case of multiple authors, you will be entitled to avail discount of 10%.

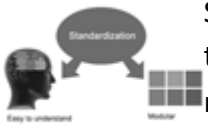
Once FARSB title is accorded, the Fellow is authorized to organize a symposium/seminar/conference on behalf of Global Journal Incorporation (USA). The Fellow can also participate in conference/seminar/symposium organized by another institution as representative of Global Journal. In both the cases, it is mandatory for him to discuss with us and obtain our consent.

You may join as member of the Editorial Board of Global Journals Incorporation (USA) after successful completion of three years as Fellow and as Peer Reviewer. In addition, it is also desirable that you should organize seminar/symposium/conference at least once.

We shall provide you intimation regarding launching of e-version of journal of your stream time to time.This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.

The FARSS can go through standards of OARS. You can also play vital role if you have any suggestions so that proper amendment can take place to improve the same for the benefit of entire research community.

As FARSS, you will be given a renowned, secure and free professional email address with 100 GB of space e.g. johnhall@globaljournals.org. This will include Webmail, Spam Assassin, Email Forwarders,Auto-Responders, Email Delivery Route tracing, etc.



The FARSS will be eligible for a free application of standardization of their researches. Standardization of research will be subject to acceptability within stipulated norms as the next step after publishing in a journal. We shall depute a team of specialized research professionals who will render their services for elevating your researches to next higher level, which is worldwide open standardization.

The FARSS member can apply for grading and certification of standards of their educational and Institutional Degrees to Open Association of Research, Society U.S.A. Once you are designated as FARSS, you may send us a scanned copy of all of your credentials. OARS will verify, grade and certify them. This will be based on your academic records, quality of research papers published by you, and some more criteria. After certification of all your credentials by OARS, they will be published on your Fellow Profile link on website https://associationofresearch.org which will be helpful to upgrade the dignity.

The FARSS members can avail the benefits of free research podcasting in Global Research Radio with their research documents. After publishing the work, (including published elsewhere worldwide with proper authorization) you can upload your research paper with your recorded voice or you can utilize chargeable services of our professional RJs to record your paper in their voice on request.

The FARSS member also entitled to get the benefits of free research podcasting of their research documents through video clips. We can also streamline your conference videos and display your slides/ online slides and online research video clips at reasonable charges, on request.

© Copyright by Global Journals Inc.(US)| Guidelines Handbook

The FARSS is eligible to earn from sales proceeds of his/her researches/reference/review Books or literature, while publishing with Global Journals. The FARSS can decide whether he/she would like to publish his/her research in a closed manner. In this case, whenever readers purchase that individual research paper for reading, maximum 60% of its profit earned as royalty by Global Journals, will be credited to his/her bank account. The entire entitled amount will be credited to his/her bank account exceeding limit of minimum fixed balance. There is no minimum time limit for collection. The FARSS member can decide its price and we can help in making the right decision.

The FARSS member is eligible to join as a paid peer reviewer at Global Journals Incorporation (USA) and can get remuneration of 15% of author fees, taken from the author of a respective paper. After reviewing 5 or more papers you can request to transfer the amount to your bank account.

MEMBER OF ASSOCIATION OF RESEARCH SOCIETY IN SCIENCE (MARSS)

The ' MARSS ' title is accorded to a selected professional after the approval of the Editor-in-Chief / Editorial Board Members/Dean.

The “MARSS” is a dignified ornament which is accorded to a person’s name viz. Dr. John E. Hall, Ph.D., MARSS or William Walldroff, M.S., MARSS.

MARSS accrediting is an honor. It authenticates your research activities. After becoming MARSS, you can add 'MARSS' title with your name as you use this recognition as additional suffix to your status. This will definitely enhance and add more value and repute to your name. You may use it on your professional Counseling Materials such as CV, Resume, Visiting Card and Name Plate etc.

The following benefitscan be availed by you only for next three years from the date of certification.

MARSS designated members are entitled to avail a 25% discount while publishing their research papers (of a single author) in Global Journals Inc., if the same is accepted by our Editorial Board and Peer Reviewers. If you are a main author or co-author of a group of authors, you will get discount of 10%.

As MARSS, you will be given a renowned, secure and free professional email address with 30 GB of space e.g. johnhall@globaljournals.org. This will include Webmail, Spam Assassin, Email Forwarders,Auto-Responders, Email Delivery Route tracing, etc.

We shall provide you intimation regarding launching of e-version of journal of your stream time to time.This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.

The MARSS member can apply for approval, grading and certification of standards of their educational and Institutional Degrees to Open Association of Research, Society U.S.A.

Once you are designated as MARSS, you may send us a scanned copy of all of your credentials. OARS will verify, grade and certify them. This will be based on your academic records, quality of research papers published by you, and some more criteria.

It is mandatory to read all terms and conditions carefully.

Auxiliary Memberships

Institutional Fellow of Global Journals Incorporation (USA)-OARS (USA)

Global Journals Incorporation (USA) is accredited by Open Association of Research Society, U.S.A (OARS) and in turn, affiliates research institutions as "Institutional Fellow of Open Association of Research Society" (IFOARS).

The "FARSC" is a dignified title which is accorded to a person's name viz. Dr. John E. Hall, Ph.D., FARSC or William Walldroff, M.S., FARSC.

The IFOARS institution is entitled to form a Board comprised of one Chairperson and three to five board members preferably from different streams. The Board will be recognized as "Institutional Board of Open Association of Research Society"-(IBOARS).

The Institute will be entitled to following benefits:

The IBOARS can initially review research papers of their institute and recommend them to publish with respective journal of Global Journals. It can also review the papers of other institutions after obtaining our consent. The second review will be done by peer reviewer of Global Journals Incorporation (USA) The Board is at liberty to appoint a peer reviewer with the approval of chairperson after consulting us.

The author fees of such paper may be waived off up to 40%.

The Global Journals Incorporation (USA) at its discretion can also refer double blind peer reviewed paper at their end to the board for the verification and to get recommendation for final stage of acceptance of publication.

The IBOARS can organize symposium/seminar/conference in their country on behalf of Global Journals Incorporation (USA)-OARS (USA). The terms and conditions can be discussed separately.

The Board can also play vital role by exploring and giving valuable suggestions regarding the Standards of "Open Association of Research Society, U.S.A (OARS)" so that proper amendment can take place for the benefit of entire research community. We shall provide details of particular standard only on receipt of request from the Board.

The board members can also join us as Individual Fellow with 40% discount on total fees applicable to Individual Fellow. They will be entitled to avail all the benefits as declared. Please visit Individual Fellow-sub menu of GlobalJournals.org to have more relevant details.

© Copyright by Global Journals Inc.(US) | Guidelines Handbook

We shall provide you intimation regarding launching of e-version of journal of your stream time to time. This may be utilized in your library for the enrichment of knowledge of your students as well as it can also be helpful for the concerned faculty members.

After nomination of your institution as "Institutional Fellow" and constantly functioning successfully for one year, we can consider giving recognition to your institute to function as Regional/Zonal office on our behalf.
The board can also take up the additional allied activities for betterment after our consultation.

The following entitlements are applicable to individual Fellows:

Open Association of Research Society, U.S.A (OARS) By-laws states that an individual Fellow may use the designations as applicable, or the corresponding initials. The Credentials of individual Fellow and Associate designations signify that the individual has gained knowledge of the fundamental concepts. One is magnanimous and proficient in an expertise course covering the professional code of conduct, and follows recognized standards of practice.

Open Association of Research Society (US)/ Global Journals Incorporation (USA), as described in Corporate Statements, are educational, research publishing and professional membership organizations. Achieving our individual Fellow or Associate status is based mainly on meeting stated educational research requirements.

Disbursement of 40% Royalty earned through Global Journals : Researcher = 50%, Peer Reviewer = 37.50%, Institution = 12.50% E.g. Out of 40%, the 20% benefit should be passed on to researcher, 15 % benefit towards remuneration should be given to a reviewer and remaining 5% is to be retained by the institution.

We shall provide print version of 12 issues of any three journals [as per your requirement] out of our 38 journals worth $ 2376 USD.

Other:

The individual Fellow and Associate designations accredited by Open Association of Research Society (US) credentials signify guarantees following achievements:

- The professional accredited with Fellow honor, is entitled to various benefits viz. name, fame, honor, regular flow of income, secured bright future, social status etc.

- In addition to above, if one is single author, then entitled to 40% discount on publishing research paper and can get 10%discount if one is co-author or main author among group of authors.
- The Fellow can organize symposium/seminar/conference on behalf of Global Journals Incorporation (USA) and he/she can also attend the same organized by other institutes on behalf of Global Journals.
- The Fellow can become member of Editorial Board Member after completing 3yrs.
- The Fellow can earn 60% of sales proceeds from the sale of reference/review books/literature/publishing of research paper.
- Fellow can also join as paid peer reviewer and earn 15% remuneration of author charges and can also get an opportunity to join as member of the Editorial Board of Global Journals Incorporation (USA)
- • This individual has learned the basic methods of applying those concepts and techniques to common challenging situations. This individual has further demonstrated an in–depth understanding of the application of suitable techniques to a particular area of research practice.

Note :

"

- In future, if the board feels the necessity to change any board member, the same can be done with the consent of the chairperson along with anyone board member without our approval.
- In case, the chairperson needs to be replaced then consent of 2/3rd board members are required and they are also required to jointly pass the resolution copy of which should be sent to us. In such case, it will be compulsory to obtain our approval before replacement.
- In case of "Difference of Opinion [if any]" among the Board members, our decision will be final and binding to everyone. "

Process of submission of Research Paper

The Area or field of specialization may or may not be of any category as mentioned in 'Scope of Journal' menu of the GlobalJournals.org website. There are 37 Research Journal categorized with Six parental Journals GJCST, GJMR, GJRE, GJMBR, GJSFR, GJHSS. For Authors should prefer the mentioned categories. There are three widely used systems UDC, DDC and LCC. The details are available as 'Knowledge Abstract' at Home page. The major advantage of this coding is that, the research work will be exposed to and shared with all over the world as we are being abstracted and indexed worldwide.

The paper should be in proper format. The format can be downloaded from first page of 'Author Guideline' Menu. The Author is expected to follow the general rules as mentioned in this menu. The paper should be written in MS-Word Format (*.DOC,*.DOCX).

The Author can submit the paper either online or offline. The authors should prefer online submission.Online Submission: There are three ways to submit your paper:

(A) (I) First, register yourself using top right corner of Home page then Login. If you are already registered, then login using your username and password.

(II) Choose corresponding Journal.

(III) Click 'Submit Manuscript'. Fill required information and Upload the paper.

(B) If you are using Internet Explorer, then Direct Submission through Homepage is also available.

(C) If these two are not conveninet , and then email the paper directly to dean@globaljournals.org.

Offline Submission: Author can send the typed form of paper by Post. However, online submission should be preferred.

Preferred Author Guidelines

MANUSCRIPT STYLE INSTRUCTION (Must be strictly followed)

Page Size: 8.27" X 11'"

- Left Margin: 0.65
- Right Margin: 0.65
- Top Margin: 0.75
- Bottom Margin: 0.75
- Font type of all text should be Swis 721 Lt BT.
- Paper Title should be of Font Size 24 with one Column section.
- Author Name in Font Size of 11 with one column as of Title.
- Abstract Font size of 9 Bold, "Abstract" word in Italic Bold.
- Main Text: Font size 10 with justified two columns section
- Two Column with Equal Column with of 3.38 and Gaping of .2
- First Character must be three lines Drop capped.
- Paragraph before Spacing of 1 pt and After of 0 pt.
- Line Spacing of 1 pt
- Large Images must be in One Column
- Numbering of First Main Headings (Heading 1) must be in Roman Letters, Capital Letter, and Font Size of 10.
- Numbering of Second Main Headings (Heading 2) must be in Alphabets, Italic, and Font Size of 10.

You can use your own standard format also.
Author Guidelines:

1. General,

2. Ethical Guidelines,

3. Submission of Manuscripts,

4. Manuscript's Category,

5. Structure and Format of Manuscript,

6. After Acceptance.

1. GENERAL

Before submitting your research paper, one is advised to go through the details as mentioned in following heads. It will be beneficial, while peer reviewer justify your paper for publication.

Scope

The Global Journals Inc. (US) welcome the submission of original paper, review paper, survey article relevant to the all the streams of Philosophy and knowledge. The Global Journals Inc. (US) is parental platform for Global Journal of Computer Science and Technology, Researches in Engineering, Medical Research, Science Frontier Research, Human Social Science, Management, and Business organization. The choice of specific field can be done otherwise as following in Abstracting and Indexing Page on this Website. As the all Global

Journals Inc. (US) are being abstracted and indexed (in process) by most of the reputed organizations. Topics of only narrow interest will not be accepted unless they have wider potential or consequences.

2. ETHICAL GUIDELINES

Authors should follow the ethical guidelines as mentioned below for publication of research paper and research activities.

Papers are accepted on strict understanding that the material in whole or in part has not been, nor is being, considered for publication elsewhere. If the paper once accepted by Global Journals Inc. (US) and Editorial Board, will become the copyright of the Global Journals Inc. (US).

Authorship: The authors and coauthors should have active contribution to conception design, analysis and interpretation of findings. They should critically review the contents and drafting of the paper. All should approve the final version of the paper before submission

The Global Journals Inc. (US) follows the definition of authorship set up by the Global Academy of Research and Development. According to the Global Academy of R&D authorship, criteria must be based on:

1) Substantial contributions to conception and acquisition of data, analysis and interpretation of the findings.

2) Drafting the paper and revising it critically regarding important academic content.

3) Final approval of the version of the paper to be published.

All authors should have been credited according to their appropriate contribution in research activity and preparing paper. Contributors who do not match the criteria as authors may be mentioned under Acknowledgement.

Acknowledgements: Contributors to the research other than authors credited should be mentioned under acknowledgement. The specifications of the source of funding for the research if appropriate can be included. Suppliers of resources may be mentioned along with address.

Appeal of Decision: The Editorial Board's decision on publication of the paper is final and cannot be appealed elsewhere.

Permissions: It is the author's responsibility to have prior permission if all or parts of earlier published illustrations are used in this paper.

Please mention proper reference and appropriate acknowledgements wherever expected.

If all or parts of previously published illustrations are used, permission must be taken from the copyright holder concerned. It is the author's responsibility to take these in writing.

Approval for reproduction/modification of any information (including figures and tables) published elsewhere must be obtained by the authors/copyright holders before submission of the manuscript. Contributors (Authors) are responsible for any copyright fee involved.

3. SUBMISSION OF MANUSCRIPTS

Manuscripts should be uploaded via this online submission page. The online submission is most efficient method for submission of papers, as it enables rapid distribution of manuscripts and consequently speeds up the review procedure. It also enables authors to know the status of their own manuscripts by emailing us. Complete instructions for submitting a paper is available below.

Manuscript submission is a systematic procedure and little preparation is required beyond having all parts of your manuscript in a given format and a computer with an Internet connection and a Web browser. Full help and instructions are provided on-screen. As an author, you will be prompted for login and manuscript details as Field of Paper and then to upload your manuscript file(s) according to the instructions.

To avoid postal delays, all transaction is preferred by e-mail. A finished manuscript submission is confirmed by e-mail immediately and your paper enters the editorial process with no postal delays. When a conclusion is made about the publication of your paper by our Editorial Board, revisions can be submitted online with the same procedure, with an occasion to view and respond to all comments.

Complete support for both authors and co-author is provided.

4. MANUSCRIPT'S CATEGORY

Based on potential and nature, the manuscript can be categorized under the following heads:

Original research paper: Such papers are reports of high-level significant original research work.

Review papers: These are concise, significant but helpful and decisive topics for young researchers.

Research articles: These are handled with small investigation and applications

Research letters: The letters are small and concise comments on previously published matters.

5.STRUCTURE AND FORMAT OF MANUSCRIPT

The recommended size of original research paper is less than seven thousand words, review papers fewer than seven thousands words also.Preparation of research paper or how to write research paper, are major hurdle, while writing manuscript. The research articles and research letters should be fewer than three thousand words, the structure original research paper; sometime review paper should be as follows:

Papers: These are reports of significant research (typically less than 7000 words equivalent, including tables, figures, references), and comprise:

(a)Title should be relevant and commensurate with the theme of the paper.

(b) A brief Summary, "Abstract" (less than 150 words) containing the major results and conclusions.

(c) Up to ten keywords, that precisely identifies the paper's subject, purpose, and focus.

(d) An Introduction, giving necessary background excluding subheadings; objectives must be clearly declared.

(e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition; sources of information must be given and numerical methods must be specified by reference, unless non-standard.

(f) Results should be presented concisely, by well-designed tables and/or figures; the same data may not be used in both; suitable statistical data should be given. All data must be obtained with attention to numerical detail in the planning stage. As reproduced design has been recognized to be important to experiments for a considerable time, the Editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned un-refereed;

(g) Discussion should cover the implications and consequences, not just recapitulating the results; conclusions should be summarizing.

(h) Brief Acknowledgements.

(i) References in the proper form.

Authors should very cautiously consider the preparation of papers to ensure that they communicate efficiently. Papers are much more likely to be accepted, if they are cautiously designed and laid out, contain few or no errors, are summarizing, and be conventional to the approach and instructions. They will in addition, be published with much less delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and to make suggestions to improve briefness.

It is vital, that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

Format

Language: The language of publication is UK English. Authors, for whom English is a second language, must have their manuscript efficiently edited by an English-speaking person before submission to make sure that, the English is of high excellence. It is preferable, that manuscripts should be professionally edited.

Standard Usage, Abbreviations, and Units: Spelling and hyphenation should be conventional to The Concise Oxford English Dictionary. Statistics and measurements should at all times be given in figures, e.g. 16 min, except for when the number begins a sentence. When the number does not refer to a unit of measurement it should be spelt in full unless, it is 160 or greater.

Abbreviations supposed to be used carefully. The abbreviated name or expression is supposed to be cited in full at first usage, followed by the conventional abbreviation in parentheses.

Metric SI units are supposed to generally be used excluding where they conflict with current practice or are confusing. For illustration, 1.4 l rather than 1.4 × 10-3 m3, or 4 mm somewhat than 4 × 10-3 m. Chemical formula and solutions must identify the form used, e.g. anhydrous or hydrated, and the concentration must be in clearly defined units. Common species names should be followed by underlines at the first mention. For following use the generic name should be constricted to a single letter, if it is clear.

Structure

All manuscripts submitted to Global Journals Inc. (US), ought to include:

Title: The title page must carry an instructive title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) wherever the work was carried out. The full postal address in addition with the e-mail address of related author must be given. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining and indexing.

Abstract, used in Original Papers and Reviews:

Optimizing Abstract for Search Engines

Many researchers searching for information online will use search engines such as Google, Yahoo or similar. By optimizing your paper for search engines, you will amplify the chance of someone finding it. This in turn will make it more likely to be viewed and/or cited in a further work. Global Journals Inc. (US) have compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Key Words

A major linchpin in research work for the writing research paper is the keyword search, which one will employ to find both library and Internet resources.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy and planning a list of possible keywords and phrases to try.

Search engines for most searches, use Boolean searching, which is somewhat different from Internet searches. The Boolean search uses "operators," words (and, or, not, and near) that enable you to expand or narrow your affords. Tips for research paper while preparing research paper are very helpful guideline of research paper.

Choice of key words is first tool of tips to write research paper. Research paper writing is an art.A few tips for deciding as strategically as possible about keyword search:

- One should start brainstorming lists of possible keywords before even begin searching. Think about the most important concepts related to research work. Ask, "What words would a source have to include to be truly valuable in research paper?" Then consider synonyms for the important words.
- It may take the discovery of only one relevant paper to let steer in the right keyword direction because in most databases, the keywords under which a research paper is abstracted are listed with the paper.
- One should avoid outdated words.

Keywords are the key that opens a door to research work sources. Keyword searching is an art in which researcher's skills are bound to improve with experience and time.

Numerical Methods: Numerical methods used should be clear and, where appropriate, supported by references.

Acknowledgements: Please make these as concise as possible.

References

References follow the Harvard scheme of referencing. References in the text should cite the authors' names followed by the time of their publication, unless there are three or more authors when simply the first author's name is quoted followed by et al. unpublished work has to only be cited where necessary, and only in the text. Copies of references in press in other journals have to be supplied with submitted typescripts. It is necessary that all citations and references be carefully checked before submission, as mistakes or omissions will cause delays.

References to information on the World Wide Web can be given, but only if the information is available without charge to readers on an official site. Wikipedia and Similar websites are not allowed where anyone can change the information. Authors will be asked to make available electronic copies of the cited information for inclusion on the Global Journals Inc. (US) homepage at the judgment of the Editorial Board.

The Editorial Board and Global Journals Inc. (US) recommend that, citation of online-published papers and other material should be done via a DOI (digital object identifier). If an author cites anything, which does not have a DOI, they run the risk of the cited material not being noticeable.

The Editorial Board and Global Journals Inc. (US) recommend the use of a tool such as Reference Manager for reference management and formatting.

Tables, Figures and Figure Legends

Tables: Tables should be few in number, cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g. Table 4, a self-explanatory caption and be on a separate sheet. Vertical lines should not be used.

Figures: Figures are supposed to be submitted as separate files. Always take in a citation in the text for each figure using Arabic numbers, e.g. Fig. 4. Artwork must be submitted online in electronic form by e-mailing them.

Preparation of Electronic Figures for Publication

Even though low quality images are sufficient for review purposes, print publication requires high quality images to prevent the final product being blurred or fuzzy. Submit (or e-mail) EPS (line art) or TIFF (halftone/photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Do not use pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings) in relation to the imitation size. Please give the data for figures in black and white or submit a Color Work Agreement Form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution (at final image size) ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs) : >350 dpi; figures containing both halftone and line images: >650 dpi.

Color Charges: It is the rule of the Global Journals Inc. (US) for authors to pay the full cost for the reproduction of their color artwork. Hence, please note that, if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a color work agreement form before your paper can be published.

Figure Legends: Self-explanatory legends of all figures should be incorporated separately under the heading 'Legends to Figures'. In the full-text online edition of the journal, figure legends may possibly be truncated in abbreviated links to the full screen version. Therefore, the first 100 characters of any legend should notify the reader, about the key aspects of the figure.

6. AFTER ACCEPTANCE

Upon approval of a paper for publication, the manuscript will be forwarded to the dean, who is responsible for the publication of the Global Journals Inc. (US).

6.1 Proof Corrections

The corresponding author will receive an e-mail alert containing a link to a website or will be attached. A working e-mail address must therefore be provided for the related author.

Acrobat Reader will be required in order to read this file. This software can be downloaded

(Free of charge) from the following website:

www.adobe.com/products/acrobat/readstep2.html. This will facilitate the file to be opened, read on screen, and printed out in order for any corrections to be added. Further instructions will be sent with the proof.

Proofs must be returned to the dean at dean@globaljournals.org within three days of receipt.

As changes to proofs are costly, we inquire that you only correct typesetting errors. All illustrations are retained by the publisher. Please note that the authors are responsible for all statements made in their work, including changes made by the copy editor.

6.2 Early View of Global Journals Inc. (US) (Publication Prior to Print)

The Global Journals Inc. (US) are enclosed by our publishing's Early View service. Early View articles are complete full-text articles sent in advance of their publication. Early View articles are absolute and final. They have been completely reviewed, revised and edited for publication, and the authors' final corrections have been incorporated. Because they are in final form, no changes can be made after sending them. The nature of Early View articles means that they do not yet have volume, issue or page numbers, so Early View articles cannot be cited in the conventional way.

6.3 Author Services

Online production tracking is available for your article through Author Services. Author Services enables authors to track their article - once it has been accepted - through the production process to publication online and in print. Authors can check the status of their articles online and choose to receive automated e-mails at key stages of production. The authors will receive an e-mail with a unique link that enables them to register and have their article automatically added to the system. Please ensure that a complete e-mail address is provided when submitting the manuscript.

6.4 Author Material Archive Policy

Please note that if not specifically requested, publisher will dispose off hardcopy & electronic information submitted, after the two months of publication. If you require the return of any information submitted, please inform the Editorial Board or dean as soon as possible.

6.5 Offprint and Extra Copies

A PDF offprint of the online-published article will be provided free of charge to the related author, and may be distributed according to the Publisher's terms and conditions. Additional paper offprint may be ordered by emailing us at: editor@globaljournals.org .

Before start writing a good quality Computer Science Research Paper, let us first understand what is Computer Science Research Paper? So, Computer Science Research Paper is the paper which is written by professionals or scientists who are associated to Computer Science and Information Technology, or doing research study in these areas. If you are novel to this field then you can consult about this field from your supervisor or guide.

TECHNIQUES FOR WRITING A GOOD QUALITY RESEARCH PAPER:

1. Choosing the topic: In most cases, the topic is searched by the interest of author but it can be also suggested by the guides. You can have several topics and then you can judge that in which topic or subject you are finding yourself most comfortable. This can be done by asking several questions to yourself, like Will I be able to carry our search in this area? Will I find all necessary recourses to accomplish the search? Will I be able to find all information in this field area? If the answer of these types of questions will be "Yes" then you can choose that topic. In most of the cases, you may have to conduct the surveys and have to visit several places because this field is related to Computer Science and Information Technology. Also, you may have to do a lot of work to find all rise and falls regarding the various data of that subject. Sometimes, detailed information plays a vital role, instead of short information.

2. Evaluators are human: First thing to remember that evaluators are also human being. They are not only meant for rejecting a paper. They are here to evaluate your paper. So, present your Best.

3. Think Like Evaluators: If you are in a confusion or getting demotivated that your paper will be accepted by evaluators or not, then think and try to evaluate your paper like an Evaluator. Try to understand that what an evaluator wants in your research paper and automatically you will have your answer.

4. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

5. Ask your Guides: If you are having any difficulty in your research, then do not hesitate to share your difficulty to your guide (if you have any). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work then ask the supervisor to help you with the alternative. He might also provide you the list of essential readings.

6. Use of computer is recommended: As you are doing research in the field of Computer Science, then this point is quite obvious.

7. Use right software: Always use good quality software packages. If you are not capable to judge good software then you can lose quality of your paper unknowingly. There are various software programs available to help you, which you can get through Internet.

8. Use the Internet for help: An excellent start for your paper can be by using the Google. It is an excellent search engine, where you can have your doubts resolved. You may also read some answers for the frequent question how to write my research paper or find model research paper. From the internet library you can download books. If you have all required books make important reading selecting and analyzing the specified information. Then put together research paper sketch out.

9. Use and get big pictures: Always use encyclopedias, Wikipedia to get pictures so that you can go into the depth.

10. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right! It is a good habit, which helps to not to lose your continuity. You should always use bookmarks while searching on Internet also, which will make your search easier.

11. Revise what you wrote: When you write anything, always read it, summarize it and then finalize it.

12. Make all efforts: Make all efforts to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in introduction, that what is the need of a particular research paper. Polish your work by good skill of writing and always give an evaluator, what he wants.

13. Have backups: When you are going to do any important thing like making research paper, you should always have backup copies of it either in your computer or in paper. This will help you to not to lose any of your important.

14. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several and unnecessary diagrams will degrade the quality of your paper by creating "hotchpotch." So always, try to make and include those diagrams, which are made by your own to improve readability and understandability of your paper.

15. Use of direct quotes: When you do research relevant to literature, history or current affairs then use of quotes become essential but if study is relevant to science then use of quotes is not preferable.

16. Use proper verb tense: Use proper verb tenses in your paper. Use past tense, to present those events that happened. Use present tense to indicate events that are going on. Use future tense to indicate future happening events. Use of improper and wrong tenses will confuse the evaluator. Avoid the sentences that are incomplete.

17. Never use online paper: If you are getting any paper on Internet, then never use it as your research paper because it might be possible that evaluator has already seen it or maybe it is outdated version.

18. Pick a good study spot: To do your research studies always try to pick a spot, which is quiet. Every spot is not for studies. Spot that suits you choose it and proceed further.

19. Know what you know: Always try to know, what you know by making objectives. Else, you will be confused and cannot achieve your target.

20. Use good quality grammar: Always use a good quality grammar and use words that will throw positive impact on evaluator. Use of good quality grammar does not mean to use tough words, that for each word the evaluator has to go through dictionary. Do not start sentence with a conjunction. Do not fragment sentences. Eliminate one-word sentences. Ignore passive voice. Do not ever use a big word when a diminutive one would suffice. Verbs have to be in agreement with their subjects. Prepositions are not expressions to finish sentences with. It is incorrect to ever divide an infinitive. Avoid clichés like the disease. Also, always shun irritating alliteration. Use language that is simple and straight forward. put together a neat summary.

21. Arrangement of information: Each section of the main body should start with an opening sentence and there should be a changeover at the end of the section. Give only valid and powerful arguments to your topic. You may also maintain your arguments with records.

22. Never start in last minute: Always start at right time and give enough time to research work. Leaving everything to the last minute will degrade your paper and spoil your work.

23. Multitasking in research is not good: Doing several things at the same time proves bad habit in case of research activity. Research is an area, where everything has a particular time slot. Divide your research work in parts and do particular part in particular time slot.

24. Never copy others' work: Never copy others' work and give it your name because if evaluator has seen it anywhere you will be in trouble.

25. Take proper rest and food: No matter how many hours you spend for your research activity, if you are not taking care of your health then all your efforts will be in vain. For a quality research, study is must, and this can be done by taking proper rest and food.

26. Go for seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

27. Refresh your mind after intervals: Try to give rest to your mind by listening to soft music or by sleeping in intervals. This will also improve your memory.

28. Make colleagues: Always try to make colleagues. No matter how sharper or intelligent you are, if you make colleagues you can have several ideas, which will be helpful for your research.

29. Think technically: Always think technically. If anything happens, then search its reasons, its benefits, and demerits.

30. Think and then print: When you will go to print your paper, notice that tables are not be split, headings are not detached from their descriptions, and page sequence is maintained.

31. Adding unnecessary information: Do not add unnecessary information, like, I have used MS Excel to draw graph. Do not add irrelevant and inappropriate material. These all will create superfluous. Foreign terminology and phrases are not apropos. One should NEVER take a broad view. Analogy in script is like feathers on a snake. Not at all use a large word when a very small one would be sufficient. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Amplification is a billion times of inferior quality than sarcasm.

32. Never oversimplify everything: To add material in your research paper, never go for oversimplification. This will definitely irritate the evaluator. Be more or less specific. Also too, by no means, ever use rhythmic redundancies. Contractions aren't essential and shouldn't be there used. Comparisons are as terrible as clichés. Give up ampersands and abbreviations, and so on. Remove commas, that are, not necessary. Parenthetical words however should be together with this in commas. Understatement is all the time the complete best way to put onward earth-shaking thoughts. Give a detailed literary review.

33. Report concluded results: Use concluded results. From raw data, filter the results and then conclude your studies based on measurements and observations taken. Significant figures and appropriate number of decimal places should be used. Parenthetical remarks are prohibitive. Proofread carefully at final stage. In the end give outline to your arguments. Spot out perspectives of further study of this subject. Justify your conclusion by at the bottom of them with sufficient justifications and examples.

34. After conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print to the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects in your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form, which is presented in the guidelines using the template.
- Please note the criterion for grading the final paper by peer-reviewers.

Final Points:

A purpose of organizing a research paper is to let people to interpret your effort selectively. The journal requires the following sections, submitted in the order listed, each section to start on a new page.

The introduction will be compiled from reference matter and will reflect the design processes or outline of basis that direct you to make study. As you will carry out the process of study, the method and process section will be constructed as like that. The result segment will show related statistics in nearly sequential order and will direct the reviewers next to the similar intellectual paths throughout the data that you took to carry out your study. The discussion section will provide understanding of the data and projections as to the implication of the results. The use of good quality references all through the paper will give the effort trustworthiness by representing an alertness of prior workings.

Writing a research paper is not an easy job no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record keeping are the only means to make straightforward the progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear

· Adhere to recommended page limits

Mistakes to evade

- Insertion a title at the foot of a page with the subsequent text on the next page
- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

· Use standard writing style including articles ("a", "the," etc.)

· Keep on paying attention on the research topic of the paper

· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

· Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives

· Shun use of extra pictures - include only those figures essential to presenting results

Title Page:

Choose a revealing title. It should be short. It should not have non-standard acronyms or abbreviations. It should not exceed two printed lines. It should include the name(s) and address (es) of all authors.

Abstract:

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing references at this point.

An abstract is a brief distinct paragraph summary of finished work or work in development. In a minute or less a reviewer can be taught the foundation behind the study, common approach to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study, with the subsequent elements in any summary. Try to maintain the initial two items to no more than one ruling each.

- Reason of the study - theory, overall issue, purpose
- Fundamental goal
- To the point depiction of the research
- Consequences, including definite statistics - if the consequences are quantitative in nature, account quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
- As a outline of job done, it is always written in past tense
- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else

Introduction:

The **Introduction** should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled the declared objectives.

Approach:

- Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done.
- Sort out your thoughts; manufacture one key point with every section. If you make the four points listed above, you will need a least of four paragraphs.

- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
- Shape the theory/purpose specifically - do not take a broad view.
- As always, give awareness to spelling, simplicity and correctness of sentences and phrases.

Procedures (Methods and Materials):

This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt for the least amount of information that would permit another capable scientist to spare your outcome but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section. When a technique is used that has been well described in another object, mention the specific item describing a way but draw the basic principle while stating the situation. The purpose is to text all particular resources and broad procedures, so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step by step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not particulars of each process that engaged the same methodology)
- Describe the method entirely
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures
- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in every other part of the paper - avoid familiar lists, and use full sentences.

What to keep away from

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings - save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part a entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.
- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
- Put figures and tables, appropriately numbered, in order at the end of the report
- If you desire, you may place your figures and tables properly within the text of your results part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
- Despite of position, each figure must be numbered one after the other and complete with subtitle
- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

Discussion:

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
- Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work
- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

The Administration Rules

Please carefully note down following rules and regulation before submitting your Research Paper to Global Journals Inc. (US):

Segment Draft and Final Research Paper: You have to strictly follow the template of research paper. If it is not done your paper may get rejected.

- The **major constraint** is that you must independently make all content, tables, graphs, and facts that are offered in the paper. You must write each part of the paper wholly on your own. The Peer-reviewers need to identify your own perceptive of the concepts in your own terms. NEVER extract straight from any foundation, and never rephrase someone else's analysis.

- Do not give permission to anyone else to "PROOFREAD" your manuscript.

- Methods to avoid Plagiarism is applied by us on every paper, if found guilty, you will be blacklisted by all of our collaborated research groups, your institution will be informed for this and strict legal actions will be taken immediately.)
- To guard yourself and others from possible illegal use please do not permit anyone right to use to your paper and files.

Criterion for Grading a Research Paper (Compilation) by Global Journals Inc. (US)

Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals Inc. (US).

Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

Index

A

Adenocar · 8
Aeruginosa · 8
Aspergillus · 1, 2, 6, 8
Ascidians · 1, 2, 3, 5, 6, 11, 12, 13, 14, 15

C

Caryophyllene · 1, 2
Coagulation · 12, 491

D

Diphenylethanol
Dodecanoic · 5, 7

E

Enantiomeric · 1, 2
Ethylenediamine · 493
Estuarine · 3, 11

G

Germacrene · 5, 7
Geotactic · 2, 3, 6, 7, 8, 10
Gregarious · 10, 11

H

Hermaphrodite · 4, 8

I

Isokessane · 5

J

Juvenile · 2, 11, 13, 14

P

Phellandrene · 2
Perspex ·

S

Sabinene · 1, 2, 6

save our planet

Global Journal of Science Frontier Research

Visit us on the Web at www.GlobalJournals.org | www.JournalofScience.org
or email us at helpdesk@globaljournals.org

ISSN 9755896

© Global Journals