

**IMPACT OF CLIMATE STRESS ON SELECTED MARINE
BIOTA**

by

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(2013 - 20 - 108)**

THESIS

**Submitted in partial fulfilment of the
requirements for the degree of**

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Faculty of Agriculture
Kerala Agricultural University**



**ACADEMY OF CLIMATE CHANGE EDUCATION AND RESEARCH
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2018

DECLARATION

I, Mary Agnus K. A. (2013 – 20 – 108) hereby declare that this thesis entitled **“Impact of climate stress on selected marine biota”** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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Certified that this thesis entitled “**Impact of climate stress on selected marine biota**” is a record of research work done independently by Ms. Mary Agnus K. A., under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associate ship to her.

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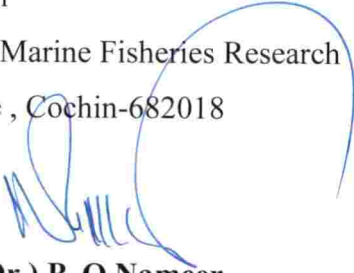
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TABLE OF CONTENTS

CHAPTER NO.	TITLE	PAGE NO.
	LIST OF TABLES	i – v
	LIST OF FIGURES	vi – vii
	LIST OF PLATES	viii
	SYMBOLS AND ABBREVIATIONS	ix – x
1	INTRODUCTION	1 – 5
2	REVIEW OF LITERATURE	6 – 21
3	MATERIALS AND METHODS	22 – 42
4	RESULTS	43 – 80
5	DISCUSSION	81 – 101
6	SUMMARY AND CONCLUSION	102 – 105
	REFERENCES	106 – 132
	ABSTRACT	

LIST OF TABLES

TABLE NO	TITLE	PAGE NO
1	Mean mortality of juvenile pearl spot (<i>Etroplus suratensis</i>) on sudden exposure to 40 ppt salinity and subsequent revival in ambient salinity (10 ppt).	44
2	Mean mortality of juvenile pearl spot (<i>Etroplus suratensis</i>) on sudden exposure to 45 ppt salinity and subsequent revival in ambient salinity (10 ppt).	44
3	Analysis of variance with salinity, time, interactions and survival rate of <i>Etroplus suratensis</i> .	44
4	Mean mortality of juvenile pearl spot (<i>Etroplus suratensis</i>) on sudden exposure to 42°C temperature and subsequent revival in ambient temperature 30°C.	46
5	Analysis of variance with temperature, time, interactions and survival rate of <i>Etroplus suratensis</i> .	46
6	Mean mortality of juvenile pompano (<i>Trachinotus blochii</i>) on sudden exposure to 5 ppt salinity and subsequent revival in ambient salinity (30 ppt).	48
7	Mean mortality of juvenile pompano (<i>Trachinotus blochii</i>) on sudden exposure to 10 ppt salinity and subsequent revival in ambient salinity (30 ppt).	49
8	Mean mortality of juvenile pompano (<i>Trachinotus blochii</i>) on sudden exposure to 15 ppt salinity and subsequent revival in ambient salinity (30 ppt).	49
9	Mean mortality of juvenile pompano (<i>Trachinotus blochii</i>) on sudden exposure to 40 ppt salinity and subsequent revival in ambient salinity (30 ppt).	49
10	Mean mortality of juvenile pompano (<i>Trachinotus blochii</i>) on sudden exposure to 45 ppt salinity and subsequent revival in ambient salinity (30 ppt).	50

11	Analysis of variance with salinity, time, interactions and survival rate of <i>Trachinotus blochii</i> .	50
12	Mean mortality of juvenile pompano (<i>Trachinotus blochii</i>) on sudden exposure to 36°C temperature and subsequent revival in ambient temperature (32°C).	51
13	Mean mortality of juvenile pompano (<i>Trachinotus blochii</i>) on sudden exposure to 37°C temperature and subsequent revival in ambient temperature (32°C).	51
14	Analysis of variance with temperature, time, interactions and survival rate of <i>Trachinotus blochii</i> .	52
15	Mean mortality of PL- 21 tiger prawn (<i>Penaeus monodon</i>) on sudden exposure to 40 ppt salinity and subsequent revival in ambient salinity (10 ppt).	53
16	Mean mortality of PL- 21 tiger prawn (<i>Penaeus monodon</i>) on sudden exposure to 45 ppt salinity and subsequent revival in ambient salinity (10 ppt).	53
17	Analysis of variance with salinity, time, interactions and survival rate of <i>Penaeus monodon</i> .	54
18	Mean mortality of PL- 21 tiger prawn (<i>Penaeus monodon</i>) on sudden exposure to 38°C temperature and subsequent revival in ambient temperature (30°C).	55
19	Analysis of variance with temperature, time, interactions and survival rate of <i>Penaeus monodon</i> .	55
20	Mean mortality of juvenile green mussel (<i>Perna viridis</i>) on sudden exposure to 5 ppt salinity and subsequent revival in ambient salinity (35 ppt).	57
21	Mean mortality of juvenile green mussel (<i>Perna viridis</i>) on sudden exposure to 10 ppt salinity and subsequent revival in ambient salinity (35 ppt).	57
22	Mean mortality of juvenile green mussel (<i>Perna viridis</i>) on sudden exposure to 15 ppt salinity and subsequent revival in ambient salinity (35 ppt).	57
23	Mean mortality of juvenile green mussel (<i>Perna viridis</i>) on sudden exposure to 20 ppt salinity and subsequent revival in ambient salinity (35 ppt).	58

24	Mean mortality of juvenile green mussel (<i>Perna viridis</i>) on sudden exposure to 40 ppt salinity and subsequent revival in ambient salinity (35 ppt).	58
25	Mean mortality of juvenile green mussel (<i>Perna viridis</i>) on sudden exposure to 45 ppt salinity and subsequent revival in ambient salinity (35 ppt).	58
26	Analysis of variance with salinity, time, interactions and survival rate of juvenile <i>Perna viridis</i> .	59
27	Mean mortality of juvenile green mussel (<i>Perna viridis</i>) on sudden exposure to 36°C temperature and subsequent revival in ambient temperature (32°C).	59
28	Mean mortality of juvenile green mussel (<i>Perna viridis</i>) on sudden exposure to 37°C temperature and subsequent revival in ambient temperature (32°C).	60
29	Mean mortality of juvenile green mussel (<i>Perna viridis</i>) on sudden exposure to 38°C temperature and subsequent revival in ambient temperature (32°C).	60
30	Analysis of variance in the juvenile green mussel (<i>Perna viridis</i>) under different conditions of survival rate, temperature and time.	60
31	Mean mortality of adult green mussel (<i>Perna viridis</i>) on sudden exposure to 5 ppt salinity and subsequent revival in ambient salinity (35 ppt).	61
32	Mean mortality of adult green mussel (<i>Perna viridis</i>) on sudden exposure to 10 ppt salinity and subsequent revival in ambient salinity (35 ppt).	62
33	Mean mortality of adult green mussel (<i>Perna viridis</i>) on sudden exposure to 15 ppt salinity and subsequent revival in ambient salinity (35 ppt).	62
34	Mean mortality of adult green mussel (<i>Perna viridis</i>) on sudden exposure to 20 ppt salinity and subsequent revival in ambient salinity (35 ppt).	62
35	Mean mortality of adult green mussel (<i>Perna viridis</i>) on sudden exposure to 45 ppt salinity and subsequent revival in ambient salinity (35 ppt).	63

36	Analysis of variance in the adult <i>Perna viridis</i> under different conditions of time, salinity and survival rate.	63
37	Mean mortality of adult green mussel (<i>Perna viridis</i>) on sudden exposure to 36°C temperature and subsequent revival in ambient temperature (32°C).	64
38	Mean mortality of adult green mussel (<i>Perna viridis</i>) on sudden exposure to 37°C temperature and subsequent revival in ambient temperature (32°C).	64
39	Mean mortality of adult green mussel (<i>Perna viridis</i>) on sudden exposure to 38°C temperature and subsequent revival in ambient temperature (32°C).	65
40	Analysis of variance in the adult <i>Perna viridis</i> under different conditions of temperature, time and survival rate.	65
41	Mean mortality of juvenile black clam (<i>Villorita cyprinoides</i>) on sudden exposure to 35 ppt salinity and subsequent revival in ambient salinity (10 ppt).	66
42	Mean mortality of juvenile black clam (<i>Villorita cyprinoides</i>) on sudden exposure to 40 ppt salinity and subsequent revival in ambient salinity (10 ppt).	67
43	Mean mortality of juvenile black clam (<i>Villorita cyprinoides</i>) on sudden exposure to 45 ppt salinity and subsequent revival in ambient salinity (10 ppt).	67
44	Analysis of variance in juvenile <i>Villorita cyprinoides</i> under different conditions of survival rate, salinity and time.	67
45	Mean mortality of juvenile black clam (<i>Villorita cyprinoides</i>) on sudden exposure to 42°C temperature and subsequent revival in ambient temperature (32°C).	68
46	Mean mortality of adult black clam (<i>Villorita cyprinoides</i>) on sudden exposure to 35 ppt salinity and subsequent revival in ambient salinity (10 ppt).	69
47	Mean mortality of adult black clam (<i>Villorita cyprinoides</i>) on sudden exposure to 40 ppt salinity and subsequent revival in ambient salinity (10 ppt).	69

48	Mean mortality of adult black clam (<i>Villorita cyprinoides</i>) on sudden exposure to 45 ppt salinity and subsequent revival in ambient salinity (10 ppt).	70
49	Analysis of variance in the adult <i>Villorita cyprinoides</i> under different conditions of salinity, time and survival rate.	70
50	Mean Mortality of adult black clam (<i>Villorita cyprinoides</i>) on sudden exposure to 40°C temperature and subsequent revival in ambient temperature (32°C).	71
51	Mean mortality of adult black clam (<i>Villorita cyprinoides</i>) on sudden exposure to 42°C temperature and subsequent revival in ambient temperature (32°C).	71
52	Analysis of variance in the adult <i>Villorita cyprinoides</i> under different conditions of temperature, time and survival rate.	72
53	Correlation analysis between different species and their exposure to varying salinities (5 to 45 ppt).	74
54	Correlation analysis between different species and their exposure to varying temperatures (30-42°C).	75
55	Resilience capacity of test animals in relation to temperature stress duration from 1 to 4 days as per category of mortality percentage.	76
56	Resilience capacity of test animals in relation to salinity stress duration from 1 to 4 days as per category of mortality percentage.	77

LIST OF FIGURES

FIGURE NO	TITLE	PAGE NO
1	Schematic diagram of the layout of the experimental set up for sudden exposure of mussels to salinity stress followed by revival.	25
2	Schematic diagram of the layout of the experimental set up for sudden exposure of mussels to temperature stress followed by revival.	26
3	Average percentage survival rate of different species with exposure to varying salinities (5 to 45 ppt) irrespective of time.	73
4	Average percentage survival rate of different species with exposure to varying temperature (30-42°C) irrespective of time.	75
5	Percentage survival of juvenile <i>Etroplus suratensis</i> on sudden exposure to varied salinities for short spells and exposure in ambient salinity for revival	83
6	Percentage survival of juvenile <i>Etroplus suratensis</i> on sudden exposure to varied temperature for short spells and exposure in ambient temperature for revival.	83
7	Percentage survival of juvenile <i>Trachinotus blochii</i> on sudden exposure to varied salinitie for short spells and exposure in ambient salinity for revival.	85
8	Percentage survival of juvenile <i>Trachinotus blochii</i> on sudden exposure to varied temperature for short spells and exposure in ambient temperature for revival.	85
9	Percentage survival of post larvae of <i>Penaeus monodon</i> on sudden exposure to varied salinities and exposure in ambient salinities for revival.	86

10	Percentage survival of post larvae of <i>Penaeus monodon</i> on sudden exposure to varied temperature for short spells and exposure in ambient temperature for revival.	88
11	Percentage survival of juvenile <i>Perna viridis</i> on sudden exposure to varied salinities for short spells and exposure in ambient salinities for revival.	89
12	Percentage survival of juvenile <i>Perna viridis</i> on sudden exposure to varied temperature for short spells and exposure in ambient temperature for revival.	89
13	Percentage survival of adult <i>Perna viridis</i> on sudden exposure to varied salinities for short spells and exposure in ambient salinities for revival.	91
14	Percentage survival of adult <i>Perna viridis</i> on sudden exposure to varied temperature for short spells and exposure in ambient temperature for revival.	92
15	Percentage survival of juvenile <i>Villorita cyprinoides</i> on sudden exposure to varied temperature for short spells and exposure in ambient temperature for revival.	93
16	Percentage survival of adult <i>Villorita cyprinoides</i> on sudden exposure to varied salinities for short spells and exposure in ambient salinities for revival.	93
17	Cell densities of <i>Isochrysis galbana</i> on sudden exposure to temperature (32°C) stress and during revival in 24°C.	95
18	Cell densities of <i>Isochrysis galbana</i> on sudden exposure to temperature (34°C) stress and during revival in 24°C.	95
19	Cell densities of <i>Isochrysis galbana</i> on sudden exposure to temperature (36°C) stress and during revival in 24°C.	97

LIST OF PLATES

PLATE NO	TITLE	PAGE NUMBER
1	Carbon dioxide flow regular and recorder	28
2	Recording observation on the fish <i>T. blochii</i> for weight (left) and total length (right)	34
3	A view of the part of experimental set up for salinity stress experiments	35
4	Measurement of green mussel <i>Perna viridis</i> using digital vernier calipers	36
5	A view of the live feed culture section of Marine Hatchery of CMFRI at Kochi	36
6	Measuring the black clam <i>Villorita cyprinoides</i> with digital vernier calipers	38
7	Experimental set up in algal culture lab of FEM Division (left); Haufkins flask with stock culture of <i>I. galbana</i> (right)	39
8	A view of the juvenile clown fish <i>Amphiprion percula</i> being acclimatized in the FEMD ocean acidification laboratory of CMFRI	39
9	Zooplankton culture maintained in marine hatchery of CMFRI	40
10	A view of <i>Isochrysis galbana</i> in a hemocytometer as observed under microscope (Magnification: 20X)	41
11	Fin rot disease in juvenile of <i>E. suratensis</i> which was exposed to sudden temperature increase from 30°C to 42°C	47
12	Changes in caudal fin of the <i>A. percula</i> on exposure to various pH concentrations	79
13	Changes in exoskeleton and appendages of the cladoceron on exposure to various pH concentrations	80

SYMBOLS AND ABBREVIATIONS

ACCER	Academy of Climate Change Education and Research
ANOVA	Analysis of Variance
AT	Atmospheric Temperature
cm	Centi meter
CMFRI	Central Marine Fisheries Research Institute
CO ₂	Carbon dioxide
Cp	Critical point
CRW	Coral Reef Watch
CT MAX	Critical Temperature Maximum
CT MIN	Critical Temperature Minimum
DHA	Docosahexaenoic Acid
ENSO	El Nino Southern Oscillation
EDE	Extreme daily events
FCR	Feed Conversion Ratio
FEMD	Fishery Environment Management Division
FRP	Fiber-reinforced plastic
gm	Gram
H ₂ SO ₄	Sulphuric acid
HID	human interface device
hrs	Hours
ICAR	Indian Council of Agricultural Research
IOD	Indian Ocean Dipole
IPCC	Intergovernmental Panel on Climate Change
IPO	Inter-decadal Pacific Oscillation
KAU	Kerala Agricultural University
L	Length
Log	Logarithm
MBTD	Marine Biotechnology Division

ml L ⁻¹	Milli litre / Litre
mm	Milli metre
MR	Moderately resilient
MV	Moderately vulnerable
N	Number
N	Nitrogen
NCEP	National Centre for Environmental Prediction
NIS	Nikon software
NOAA	National Oceanic and Atmospheric Administration
OA	Ocean acidification
PUFA	Poly unsaturated fatty acid
pCO ₂	Partial pressure of CO ₂
pH	Potential of hydrogen.
PL	Post larvae
ppt	Parts per thousand
PRL	Prolactin
R	Resilient
SAM	Southern Annular Mode
SHG	Self-help group
SPSS	Statistical Package for the Social Sciences
SST	Sea Surface Temperature
SD	Standard deviation
US	United States
UNFCC	United Nations Framework Convention on Climate Change
V	Vulnerable
μmol/L	Micromol / Litre

INTRODUCTION

CHAPTER 1

INTRODUCTION

Globally, climate change has been accepted as a major threat to ecosystems and mankind. The United Nations Framework Convention on Climate change (UNFCCC) in its Article 1 defines climate change as ‘ a change of climate that is attributed directly or indirectly to human activities, that alters the composition of global atmosphere which is in addition to the natural climate variability observed over comparable periods of time’ (IPCC, 2002). Apart from overexploitation and pollution, climate change has a pivotal role in the decline of fishery resources, which heavily support the food security of human population in developing countries. El Nino - Southern Oscillation (ENSO) like climatic phenomena are responsible for inter-annual variations in atmosphere and sea surface temperatures and rainfall of a region at long-term trend. Studies have also shown that the warm episodes of ENSO have increased since 1970 compared to the previous 100 years (IPCC, 2002). Pelagic fishes like sardines and anchovies have been found to be affected by the ecological changes associated with El-Nino (Lindegren *et al.*, 2018). It is now seen that the intensity of rainfall and droughts are remotely linked to such ocean atmospheric processes and these have local impacts.

The IPCC in its technical report on climate change and biodiversity (IPCC, 2002) has indicated that projected changes in climate can lead to increasing temperature, changes in precipitation, sea level rise and increased frequency and intensity of some extreme events. Such variations can affect the physical and biological processes of an ecosystem which in turn can influence the survival of species. Throughout the world extreme events have caused much concern. One of the most important consequences of these is the impact it has on communities and human life. The coastal and marine ecosystems are also affected and the livelihood of people depending on these ecosystem becomes vulnerable to extreme events, thus making developing countries more vulnerable. Extreme events like floods, cyclones and droughts create a negative impact on human life.

The India Meteorological Department was established in 1875 and the records from this department indicate that the country has witnessed several floods and droughts (De *et al.*, 2005). As per IPCC special report on extreme events by (Murray, *et al.*, 2012) , extreme events is the occurrence of a value of a weather variable above or below a threshold value near the upper (or lower) ends of the range of its observed values in a specific region. It is predicted that climate extremes in India is showing an increasing trend. Floods form one of the major extreme events in the country (Singh and Patwardhan, 2012).

Meteorologists have indicated that the extreme daily events (EDE) associated with monsoon have increased in some parts of the country (Krishnaswamy *et al.* 2015; Gosh *et al.* 2012; Ajaymohan and Rao 2008; Goswami *et al.* 2006 and Rajeevan *et al.*, 2006) There are also views that average Indian Monsoon has been declining (Kumar *et al.*, 2011 and Krishnaswamy *et al.*, 2015). At the same time, in certain parts it is reported to be increasing (Guhathakurta and Rajeevan 2008).

When such extreme events occur, the biota of the aquatic ecosystems are exposed to unexpected stress. It is usually not one factor which is responsible for this; there will be multiple stressors. The abiotic factors can be outside the normal range and the biological response to these changes determines the survival of the organism.

In India, four seasons have been recognized; winter during January and February, pre- monsoon or summer from March to May, southwest monsoon or summer monsoon from June to September and the post monsoon from October to December (De *et al.*, 2005). Of these, the southwest monsoon is the most important. Though there are different types of extreme events or natural disasters like cold wave, fog, snow storms and avalanches, hailstorm, thunderstorm and dust storms, heat wave, tropical cyclones and tidal waves, floods, heavy rain, landslides and droughts the most important as far as fisheries and mariculture along southwest coast of India is concerned are the floods and droughts.

Significant changes in precipitation during this period are known to lead to floods or droughts. When the precipitation is very strong for a number of days then the salinity in the coastal water lowers and it can lead to ingression of fresh water in the farming areas also. Such situation can affect the osmosis and other biological functions of coastal biota and their survival depends on their adaptive or resilience capacity. Similarly when the rainfall is much below normal for days together, the increase in temperature may not be tolerable to the biota. Along with this, the reduced flow in the rivers can lead to increased salinity and high temperature. Then also the coastal biota are exposed to stress.

The difference between the adaptation to climate change in general and adaptation towards extreme events is that in the former there is a longer time period, while in the latter the changes happen abruptly and the animal must be having capacity to withstand this sudden change in environmental factor. The rise in temperatures related to climate change has been found to be a gradual process and this has been found to cause extraordinary behaviour patterns in some species making them capable to adapt to temperature modifications. Some less tolerant species migrate toward the poles or to new regions, while some species disappear.

Plankton communities have a major role in the marine food web and these are expected to be highly sensitive to climate change. Ocean acidification, which is the result of growing absorption of atmospheric carbon dioxide (CO₂), has a significant effect on the marine organisms with calcareous skeletons or shells. It reduces the availability of carbonate ions required for formation of skeletons, thus making them weak and restricting their physical development (Melzner *et al.* 2009 and Christensen *et al.*, 2011). Byrne (2011) has reviewed the impacts especially the vulnerabilities of warming and acidification on the life history stages such as gametes, eggs and larvae of marine invertebrates. Since larvae are planktonic they are more vulnerable to these climate factors. If larval mortality is more then there will be recruitment failure, which would affect the fisheries.

Temperature and salinity are considered as some of the main factors affecting the survival of marine biota. There are other equally important parameters like the level of dissolved oxygen, total suspended solids, ammonia and pH which influence the survival of these organisms. As far as extreme events related to monsoon are concerned, temperature and salinity can be considered as the most important ecological variables. One typical example of impact of high temperature for short period is the bleaching of coral reefs. This is a response to extreme event.

Such impacts on other commercially important biota have been studied and are difficult to quantify due to various reasons. When the production decreases it is presumed that the reason would be environmental stress. So to understand the vulnerability of some commercially important resources the project work entitled 'Impact of climate stress on selected marine biota' was planned.

The main objectives were:

1. To study the effect of sudden variations in temperature, salinity and pH on selected marine biota.
2. To evaluate the resilience capacity of different life history stages of finfish and shellfishes to abiotic stress.

Temperature and salinity being the major abiotic parameters which change during extreme events, simulation experiments were planned and conducted by keeping these as variables.

The results of the study will be useful to aquaculture farmers to prepare and take decisions related to their farmed stock during flash floods and severe droughts. This would reduce the vulnerability of the fish farmers and help increase their preparedness to face climate change. Mari culture is one of the fastest growing industries and in India in addition to pond based shrimp farming, other types of aquaculture are becoming popular. Cage farming of finfishes and rack and rope method of mussel farming are becoming very popular in coastal communities. Since the investments are made by availing loan from Agriculture Banks, reduction in profit can affect repayment and lead to increased liability. Extreme events have

caused considerable damage to aquaculture industry. This study is planned to elucidate good scientific information based on which advisories can be given to farmers to handle their stock in case of an extreme event.

If the survival varied between one to four days, advisories can be given to the farmers to harvest the stock in the beginning itself. This decision to go in for distress harvest can be made only if we know the survival of fishes and bivalves to short term exposure to such extreme scenarios.

In addition to temperature and salinity, experiments were also planned to evaluate the effect of variation in pH on selected zooplankton and juvenile fish species to assess the possible impacts due to ocean acidification. This would help to take necessary steps to reduce its impact.

The results would also help to take decisions for improving the natural resource management programs in a better way by resolving or reducing the factors which exacerbate the impacts of extreme events.

There are different types of animals in an ecosystem, those which are sedentary or with little movement and those which are fast moving (Nekton). Sudden changes in the habitat can affect these in varied magnitudes. Similarly, some species are economically important, either as a major fishery resource or as a candidate species for aquaculture. For the present study care was taken to select the species. Considering the economic importance, six species were selected for the study; two bivalves *Perna viridis* (green mussel) and *Villorita cyprinoides* (black clam); one species of shrimp *Penaeus monodon* (tiger prawn); two species of fin fishes *Trachinotus blochii* (silver pompano) and *Etroplus suratensis* (pearl spot). Apart from these, one algal species also *Isochrysis galbana* (marine-microalgae) which is an important food during the larval stages of fishes and shellfishes was also selected. For ocean acidification simulation studies, juveniles of *Amphiprion percula* (Clown fish) and a zooplankton, *Daphnia salina* (Cladoceran) were selected.

REVIEW OF LITERATURE

CHAPTER 2

REVIEW OF LITERATURE

2.1 Stress on marine biota

2.1.1 Climate change impact on marine biota

Increase in human population and related anthropogenic activities have led to the phenomenon called climate change which in turn has been identified as one of the factors responsible for changes in sea surface temperature, ocean circulation, stratification, nutrient inputs and varying levels of dissolved oxygen content. Simultaneously there has been increase in carbon dioxide in the marine ecosystem leading to altered pH and ocean acidification. These factors affect the marine community and affect the biological process which affect the livelihoods depending on this important ecosystem.

As per the report of IPCC (2002), the increase in temperature, change in precipitation pattern, frequency and intensity, pH, water temperature, wind, dissolved carbon dioxide and salinity, combined anthropogenic pollution by nutrients and toxins can affect the water quality in estuarine and marine habitats. According to Kennedy *et al.* (2002) global climate change is a significant factor which affects the distribution of organisms and their interactions. The increase in the concentration of greenhouse gases has a major role in climate change which in turn creates a significant change on coastal ecosystems, especially estuaries and coral reefs due to environmental stress. Increased temperature and precipitation can cause ocean stratification which can affect ocean productivity.

Climate change can affect fish migration. Temperature is considered as one of the major abiotic factors influencing survival in the aquatic ecosystems (Brett, 1971). According to Freitas *et al.* (2015) an increase in sea surface temperature results in the vertical migration in cod species during the summer season. In a study conducted by Boero *et al.* (2008) the emerging biotic response in the Mediterranean sea by climate change was evaluated. They found positive impacts such as the increase in the species richness in northern and central sectors of Mediterranean sea.

The negative impacts were ecosystem changes, commercial species collapse, change in food chains, ecological consequences, and the risk of loss of habitat-forming species. Jellyfish outbreaks and anomalous phytoplankton blooms which may release toxic substances and mass mortalities of the marine biota such as shellfish is also related to climate change. Johnson and Moghari (2009) remarked that climate change which is happening on a global scale is ten times faster than the global warming. The ecological impact associated with this is different from one place to another.

Hoegh and Bruno (2010) remarked that marine ecosystems are centrally important to the biology of the planet. The annual primary production would decline by 70% at higher latitudes and also in Pacific and Indian Ocean regions. Phenology and physiology mismatches are reported in the marine ecosystem with changes in the pattern of primary production. Climate-induced shifts may affect the primary production through the upper trophic level (Doney *et al.*, 2011). The species-specific rate of body growth, feeding, special and metabolic activities are observed to vary due to climate change (Portner and Peck, 2011). Craig (2012) have observed that extinction of marine species is due to several factors, pollution, overfishing and the most relevant phenomenon climate change. As per this study, one million species are under stress. Climate change causes massive changes in the environment. The causal factors of climate change are greenhouse gases such as methane, carbon dioxide, ozone, and nitrous oxide which cause an increase in sea surface temperature, sea-level rise, salinity and decrease of pH.

Increasing trend of ocean surface temperature was observed in the study of Wernberg *et al.* (2012) and this indicates that there is increase of 2.5°C along the west coast of Australia from 1880 to 2000. Boyd (2011) established that complex environmental changes would affect all resources from primary producers to higher trophic levels and this can affect the biogeochemical process and the physiological performance of the different species. The simultaneous shift in environmental parameters reduces the organismal fitness, both in the pelagic and benthic

community and thus can alter the food web dynamics of the ocean system both in regional and global scale.

Poloczanska *et al.* (2013) found out that 81 to 83 % of species distribution, phenology, community composition, and demography were getting altered with climate change. They assessed that climate change has a relevant response to the species migration comparatively more than a terrestrial ecosystem. According to Constable *et al.* (2014), Antarctic and Southern Oceanic regions have changed during the past 30 years. The increase in temperature reduced the Antarctic krill productivity. Ocean acidity is also another factor which kills the embryos of Antarctic krill. Shifting of habitat location reduces the foraging success of species. The impact of climate change affects the autecology of polar region marine biota at a great level.

According to Popova *et al.* (2016), instead of temperature, the dynamics of the ocean may alter due to salinity, pH, and the low availability of oxygen. Ocean acidification would affect the rich niche of the marine ecosystem. Foo and Bryne (2017) observed that the climate change stressors are heritable. It would decrease the egg number and size in many taxa and length of the larval phase. The exposure of parents to environmental stressors is a key factor to assess the reproductive response of females to ocean acidification. Climate change is considered to create imbalances in the physiology and can lead to behavioral changes in the organism. It also alters the food web in a marine ecosystem. Pecl *et al.* (2017) have reported that climate change enhances the shifting of species from its current location to another regime. The unexpected consequences affect the biological communities on a large scale which would result from the rapid spread of disease degrading the entire community. Miller and Wiens (2017) has also observed that oceanic community composition has been affected due to climate change.

Globally, the impact of high sea surface temperature (SST) on corals has been a matter of concern and extensive studies have been done on the extent of bleaching and its damage. It is well known that mass coral bleaching occurs when the symbiotic algae zooxanthelle are affected by temperature stress (Jaap, 1985;

Jokiel and Coles, 1990). This happens when anomalously warm water temperatures spread over the coral reefs. Studies have shown that these bleaching events have occurred with increasing frequency and severity in recent decades (Eakin *et al.* 2010; Heron *et al.* 2016 and Hughes *et al.*, 2018). Now the preparedness to face such events has been possible by the recent developments in remote sensing and prediction modelling (Liu *et al.*, 2017).

2.1.2 Ocean acidification and its impacts on marine organisms

Ocean is considered as the absorber of CO₂, and the absorption rate is high now. Ocean acidification occurs due to the increase in the concentration of carbon dioxide in water which decreases the pH of water. The increase in the concentration of carbon dioxide after the pre-industrial period has been found to decrease the pH of seawater, which is a major threat for shelled organisms. It can negatively affect the mollusks, coral reefs, cephalopods, and crustaceans from micro to macro level and thus affect the food web of marine ecosystems. Altered pH can cause hypercapnia in marine organisms. The increase in the concentration of carbon dioxide in seawater can also be due to the interactions such as river water runoff, sediment at the bottom and land use practices which changes the calcification in soft-shelled organisms like clams (Salisbury *et al.*, 2008). Chemical changes affect the extracellular metabolic activities of the organism (Widdie Combe and Spicer, 2008). Kurihara (2008) found that ocean acidification negatively affects cleavage and larval settlement.

According to Feely *et al.* (2009), in the surface layer of oceans, pH will drop by 15%. They predicted that from 2095 onwards, the Arctic zone will be saturated with calcite. They compared the concentration of carbon dioxide in major ocean and observed that it was very high in the Northern Indian Ocean. Ries *et al.* (2009), found that the impact of ocean acidification on a range of benthic marine biota varied and was very high in shrimps, lobsters and crab. Clams, sea urchins and oysters showed varied response. This may reflect differences amongst organism to regulate pH due to the calcification. Ocean acidification is found to largely have negative impact among marine organism but in certain species, it provides growth benefits (Idso and

Ferguson 2009). According to Hoffman *et al.* (2010), deep sea zones are greatly affected by ocean acidification. These multiple stressors have a deleterious effect on biological functions of the ecosystem.

Acidification singly doesn't affect the marine organisms but other phenomena associated with the acidification show a great effect on marine biota (Hendrick *et al.*, 2010). The interplay between ocean acidification and numerous anthropogenic stressors have a cumulative effect on ocean biota and affect the marine food web (Boyd, 2011).

Effect of ocean acidification on the early life stages of mussel *Mytilus edulis* was studied by (Bechmann *et al.*, 2011). The size of the larvae under lower pH (7.6) was very small compared with control one. At the end of the experiment, they found out that the settlement of larvae (28 %) was smaller at the lower pH than the normal pH (8.2) Ocean acidification has also been found to affect the brain functioning of tropical reef species. (Branch *et al.*, 2013). As per the study of Glaspie *et al.* (2017), estuarine acidification decreases the fitness of the organism. They remarked that acidified clams had lighter shells than ambient clams.

2.1.3 Effect of temperature on marine ecosystem

According to Heilmayer *et al.* (2004), growth efficiency decreases with an increase of temperature in the Pectinid (bivalve) population and its species. The enzymatic activity decreased due to the increase in temperature. Helmuth *et al.* (2006), have indicated that the biogeographic patterns of the environment are very relevant to any organism. Gilman *et al.* (2009), found out that the thermal tolerances of intertidal species *Mytilus californianus* are vulnerable to climate change. The increase of 1°C of air and water temperature raised the body temperature by 0.07 to 0.90°C which would decrease the physiological and biological rhythm of the organism.

Tomanek (2010) has observed that the species from extreme thermal ends are extremely variable with the thermal environment, which is to a greater extent affected by climate change. Investigations by Lathlean and Minchinton (2012) have

indicated that the larval settlement of barnacle is influenced by the substratum. They observed that black color reduces the larval settlement, because that is a good absorber of heat compared with others and it affects the recruitment process.

Collodaria is a major species of phytoplankton in the oceanic system which has a negative response in the colonial form with respect to temperature. Its colonial density rapidly declines with a temperature of 21°C and 25°C (Villar *et al.*, 2018).

2.1.4 Concerns of salinity stress in marine biota

The experiments conducted by Lasker *et al.* (1972) found that, 40 ppt is the tolerance limit in *Saridiella* and *Sargo* species. The mortality rate was very high during upper and lower salinity range which also reduced the success of hatching and embryo growth. According to Koehn *et al.* (1989) the metabolic rate, reproduction, growth, net energy balance of the organisms will be altered due to stress from environment. Protein synthesis in an organism plays a crucial role in its metabolism (Hawkins, *et al.* 1988 and Kohen *et al.*, 1989). In a study conducted by Krist *et al.* (1990) it was observed that extreme salinity stress reduces the metabolic functions of marine algae, perhaps it would also reduce the formation of the cell wall, and loss of ion concentration. It was observed that there was an increase in the concentration of PRL and decrease in the concentration of thyroid hormones due to salinity stress as revealed from the study in response of *Paralichthys olivaceus* in the metamorphosis stage (Hiroi *et al.*, 1997).

The increased temperatures and variability of precipitation associated with climate change are likely to influence the size of organisms, from primary producers to top predators (Sheridan *et al.*, 2011).

According to Yang *et al.* (2016), the response of salinity and temperature in *Crassostrea gigas* from wild and farmed areas varied with temperature and salinity. Wild oysters had the ability to adapt to climate change, they could tolerate extreme temperature and salinity as compared with farmed oysters.

Low salinity and the high temperature had interactive effects in reducing larval survivorship. Sea urchin is an important ecological species which is

vulnerable to global warming. The larval stages are highly affected due to the decrease in salinity followed by precipitation (Mak *et al.*, 2018). While comparing the combined and individual stressors, in the former, interactive effect was synergistic (Folt *et al.*, 1999; Przeslawski *et al.*, 2015 and Mak *et al.*, 2018). In a short-term period also the salinity and temperature affect the blastula formation, survival, and growth of pluteus larvae (Urriago *et al.* 2016 and Mak *et al.*, 2018).

2.2 Extreme events

2.2.1 Implications of extreme events in marine biology

According to De *et al.* (2005), the impacts of extreme weather events lead to vulnerability among populations. The climatic oscillations such as El-Nino/Southern oscillation have a great role in the warming of the climate. The amalgam of several climatic oscillations is responsible for heat waves, cold waves and tropical cyclones in various parts of the world. Anthropogenic activities have a large connotation in the occurrence of extreme weather effects.

Pearcy *et al.* (1987) stated that the impact on the Alaskan coastal region during El-Nino epoch from 1982 to 1983, there was a deterioration in salmon fishes. This phenomenon diverts the biological contextual of the organisms at a great rate. High mortality was documented in the maturing stage of fishes.

According to Bakun and Broad (2003), the climatic oscillator El-Nino has a significant role in the decline of the anchovian fishery in the Peruvian coast of America. This effect increases warming in the ocean and declines the nutrient enrichment of water. El-Nino southern oscillation influence population dynamics in many species in the marine ecosystem. They found out that the strength and variability of the Leeuwin Current in Western Australia have a relevant role in the abundance of Dolphin species. With these events, the following factors also vary such as sea surface temperature, salinity and rainfall.

Climate change increases the severity of extreme events. The different climate models found out that there is an increment in the pattern of marine heat of the Central Equatorial Pacific, Pacific Northwest, North Pacific Ocean or Alaska

and Australia regions. According to Yu *et al.* (2015), the adverse climatic condition, ocean warming in the southeast Pacific Ocean off Peruvian water was observed which led where to shortage of the squid, *Dosidicus gigas*. The quick addition of heat caused the species shifting and reduction in the potential quality. El-Nino events associated with water temperature caused a negative effect on the coral reef fishes in the tropical reef system. During El-Nino period (1997-1998) it was observed that there was a rapid increase in sea surface temperature, lessening the strength of westward surface current towards the reef and chlorophyll concentration was really depressed. Conversely, there was a positive response in the La-Nina phase which was a favourable condition for fishes (Loyat *et al.*, 2011).

The greenhouse warming has a crucial role in the extreme events such as high temperature and rainfall extremes Coumou (2012). Werenberg *et al.* (2012) confirmed that the extreme climatic events alter the distribution and abundance of marine biota directly through physiological stress. It also affects the proper structure and functioning of ecosystems. The role of climatic drivers such as El -Nino southern oscillation (ENSO), Southern annular mode (SAM), Indian ocean dipole (IOD), and Indian Pacific oscillation (IPO) have a significant role in the habitat of the Australian marine environment. These drivers during different phases changed the extreme and average values of variables of sea surface temperature, salinity, wind, and thermocline depth of ocean and also shifted the species distribution (Klaer *et al.*, 2015). The above cited climatic drivers have also been cited to be responsible for extreme events, marine heat in Australia, coral bleaching in Great Barrier Reef and also flooding in Queen Island (Salinger *et al.*, 2016).

Lindegren *et al.* (2018) stated that the bottom up and top down, forcing mechanisms to affect the biological dynamics of the ocean system. The biota respond differently to major events. The Pelagic fishery is really sensitive to extreme El-Nino events. The low nutrient enrichment in the oceanic province has retarded the growth of ecosystem at various trophic levels. Poloczanska (2018) confirmed that the anomalous warming period and storms and the associated changes are the responsibility factor which diminishes the distribution and the abundance of marine

organism and the fishery resources. The influence of large El-Nino event shifts the fishing effort of the fleets in the equatorial Pacific region which reduces the fishing activity in the economic exclusive zone (Kroodsmma *et al.*, 2018 and Poloczanska, 2018).

In the Indian sub-continent, extreme events have been found to affect human populations. Most studies have been by meteorologists. Krishnaswamy *et al.* (2015) found out that the Indian Ocean Dipole and ENSO are the key drivers of the Indian monsoon. The ocean-atmosphere phenomenon influences the precipitation pattern around India at a countless rate. Not much work has been done on the impacts of extreme events on marine biota. Recently, the decline in the oil sardine fishery has been linked with high SST and low food (Kripa *et al.*, 2018).

2.3 Impacts of environmental stress on finfishes

2.3.1 *Etroplus suratensis*

Etroplus suratensis commonly known as pearl spot has been found in brackish water and even in fresh water zones (Hora and Pillay 1962). George and Sebastian (1970) have reported that pearl spot was one of the important species caught by different gears in the coastal waters of Kerala. It has been preferred over other species of the same genus by aqua-farmers because of its larger size and comparatively faster growth rate. This is farmed in ponds in several parts of India De Silva and Perera (1984). During the last two decades concerted efforts have been made to breed this fish and also to develop hatchery technologies for supplying seed to farmers. The natural population of pearl spot was found to decline (Padmakumar *et al.*, 2002). At the same time, demand for this resource has been found to be increasing and Kumar and Jain (2009) stated that this species has become popular as a candidate species for culture.

Though there are publications on the fishery and aquaculture of this species, not much work has been done on its response to environmental stress. One of the first reports on the response to stress by this species is by Menon *et al.* (1959). They have stated that in spite of the fact that the species has wide distribution the fry and

fingerlings cannot tolerate the stress while transferring them directly from sea water to freshwater. Gills of a fish are the primary organs which help the fish in osmoregulation and it balances the movement of ions (Hirose *et al.*, 2003). Evans *et al.* (2005) have stated that euryhaline fishes have very efficient osmo-regulatory mechanisms which help them to survive in wide range of salinities. Recent studies have shown that the mitochondria-rich cells in the gill epithelium are the ionocytes responsible for ion uptake in freshwater and ion secretion in seawater (Hirose *et al.*, 2003; Hwang and Lee 2007).

2.3.2 *Trachinotus blochii*

Young ones of pompano were observed in natural aquatic ecosystems with varied salinities during last century (Finucane, 1969; Perret *et al.*, 1971 and Gilbert 1986). One of the first studies on *Trachinotus carolinus* has been by Moe *et al.* (1968) where they observed that this species could survive in low salinities with acclimatization. Chervinski (1973) found that the survival of *Trachinotus ovatus* in different salinities varied. He found that there was difference in tolerance levels with and without acclimatization. Silver pompano has been identified as a candidate species for aquaculture in many parts of the world. Other species of carangids like *Trachinotus marginatus* and the Florida pompano, *Trachinotus carolinus* have been identified for farming because of their fast growth and ability to survive in different salinities (Menezes and Figueiredo, 1980; Sampaio *et al.*, 2003; Weirich and Riche, 2006). These fishes have been found to tolerate a wide range of salinities ranging between 7 to 58 ppt. Studies have shown that with acclimatization they can survive even in lower salinities (Sampaio *et al.*, 2003). Mc Master *et al.* (2006) found that *T. carolinus* had similar growth in 19 ppt and 35 ppt. However, Main *et al.* (2008) found that, *T. carolinus* reared in lower salinities had high mortality. Wills *et al.* (2007) observed varied growth and survival of the same species in different salinities. Huang *et al.* (2008) who studied the effect of different salinity levels on juveniles of *T. ovatus* juveniles found good growth and survival in 25 ppt. Chavez *et al.* (2011) has also studied growth of *T. blochii* in different salinities. More recently, Kalidas *et al.* (2012) have conducted experiments to understand the

performance of silver pompano in different salinities with a view to promote its aquaculture in the country.

2.4. Response of shrimp resources

2.4.1 Shrimp and multiple stressors

An experiment was conducted by Sandifer (1973) on the larval development of grass shrimp to study the effect of temperature and salinity. This study indicated that high temperature would decline the growth of shrimp and more variation occurred in the development of larval stages. Comparison of high and low salinities indicated that high salinity favoured the growth of shrimp and low salinity inhibited the growth of larval stages of shrimp. As per the study of Ponce *et al.* (1997), the impact of salinity, temperature and temperature-salinity linking have a more noteworthy impact in the development and survival of adolescent white shrimp (*Penaeus vannamei*). Survival was low at 20°C and higher temperature lessened the development and survival rate. According to Abraham *et al.* (2009), influence of salinity in modified extensive management practice, resulted in a high rate of stress due to the lack of oxygen. In higher saline water, the rate of vibrio production was very high. As evaluating the FCR (feed conversion ratio) and survival rate in both ponds (high and low saline ponds) high saline ponds were better than low saline ponds. Physio chemical parameters, especially temperature has a great influence on the pond dynamics.

2.5 Climatic stressors in bivalves

2.5.1 Variation limits in mussel species

Salinity and temperature (the abiotic factors) have a significant role in the settlement of the green mussel (De Bravo *et al.*, 1998). Anestis *et al.* (2007) stated that temperature has a great role in the survivorship of *Mytilus galloprovincialis* species. Temperature also affected the opening and closure of valves in this mussel. The study indicated that these species cannot survive in seawater temperature beyond 26°C. There is a drastic decline in the number of mussels when exposed to

this temperature. A study conducted by Yuan *et al.* (2010) indicated that *Mytella charruana* a new species in the Atlantic coastal region showed 90 % mortality in 0 and 45 ppt salinities. They endured in best in salinities 2 to 23 ppt. These mussels can survive over a wide range of salinity and in environment with substantial fresh or saltwater input. In different salinities, they exhibited different survival rate.

As per the study of Firth *et al.* (2011), the mortality of the green mussel over the intertidal region of the Southeastern United States was due to the extreme weather conditions. When the air temperature was below 2°C for a period of 6 hours, they found that mussels were affected. They found that during 2007 onwards the richness of mussel in that region was very high, subsequently it abruptly shrunk from 2008 to 2010. According to Wang *et al.* (2011), higher mortality of green mussel was recorded in high temperature and hypoxic conditions. During high temperature, the dissolved oxygen level was reduced. While compared with different temperature, high mortality was recorded in 30°C

Wendling *et al.* (2013) indicated that that human-induced degradation along with environmental stresses are responsible for the mortality of green mussel.

Salinity is an important environmental parameter influencing various life stages of aquatic life in coastal and marine species. The modification of calcium and carbonate in seawater prevents the shell formation in the trochophore stage of the bivalves, which is a critical factor of the existence of these species (Thomsen *et al.*, 2018). Wu *et al.* (2018), remarked that high, low salinity and low pH have a negative role in the immune system of the mussel *Mytilus coruscus*. The impact of low pH is severe than salinity change and the overall stresses is very large. Decreased pH had a tougher impact than salinity changes and the destructive impact was distended when both stressors were combined. According to Wang *et al.* (2018), the effect of thermal stress both acute cold and heat affects the physiological response of the mussel, *Perna viridis*.

Studies on effect of environmental variations on the green mussel in India are limited. Manoj and Appukuttan (2003) observed that the effect of temperature has a profound impact on the development of green mussel from settlement to its

growth. Temperature greater than 29°C is not good for larval settlement of green mussel. The study indicated that mussel larvae are sensitive to low temperatures as indicated by poor growth, settlement and spat production. According to Rajesh *et al.* (2001) salinity has a significant impact on the filtration rate on bivalves. Sasikumar *et al.* (2011) found that the physiological response of green mussel varies with environmental variability. Condition index of green mussel showed variation with riverine influence and was poor in unfavourable environmental conditions.

2.5.2 Responses of clam

Clams are mostly sedentary and can be more impacted by climate extremes. As per the study of Xiao *et al.* (2014) variation of temperature and salinity are the responsible factors for the survival of *Corbicula fluminea*. The smaller ones can adapt and have more survival capacity than larger ones. The effects of environmental factors had a significant role in the growth, survival, and metamorphosis of geoduck clam. As per the study of Wynsberge *et al.* (2018), the climatic variability has a great role on the mass mortality of giant clam in the tropical Pacific Ocean reef. The natural mortality of clams varied with the fluctuations of El-Nino.

In India, few studies have been done on the impacts of environmental variations on clam. According to Raveenderan (2011), the smaller clams are more resistant than larger clams. Organisms in lower salinity accumulated more toxic chemicals. The distribution of dissolved oxygen is inversely proportional salinity. Suja and Mohammed *et al.* (2010), have indicated that the black clam fishery in the southern part of the Vembanad Lake was declining because of the water was getting fresher, which is a threat to its landings and could cause a serious problem to the fishers. The black clam fishery did not show signs of overexploitation of the clams. The long-standing effects of the Thaneermukkom bund and industrial pollution may lead progressively to some decline in their abundances.

2.6 Effect of temperature in marine micro algae

2.6.1 *Isochrysis galbana*

Isochrysis galbana is a marine unicellular marine phytoflagellate. Kaplan *et al.* (1986) have conducted series of experiments on the influence of temperature on the production of *I.galbana*. They found that optimal temperature which gave the best yield was 27°C and temperatures higher than 32°C or lower than 19°C reduced the production. It has also been found to vary with environmental factors. Several studies have indicated that temperature affects the fatty acid composition of microalgae (Seto *et al.*, 1984; Mortensen *et al.*, 1988; Thompson *et al.*, 1992). It has been observed that the fatty acid profiles and lipid content vary between strains of *Isochrysis* (Alonso *et al.*, 1992; 1994) and that temperature affects the lipid and biochemical composition of *Isochrysis galbana* (Zhu *et al.*, 1997).

2.7 Ocean acidification

Climate change studies focusing on absorbing of carbon dioxide by oceans have reported that by the year 2100, partial pressure of CO₂ of sea water (pCO₂) will double from current levels to 750 ppm. This can lead to a lowering of surface water pH of nearly 0.4 by the end of the century, and can lead to a 50 % decrease in carbonate ion concentration (Feely *et al.*, 2004; 2008). Coastal regions like estuaries already have levels of CO₂ that are much higher than those predicted to occur by the end of the century (Frommel *et al.* 2013; McElhany and Busch 2013; Murray *et al.* 2014). The reponse of different biota to such changes has been a topic of research in several regions. The information would help to understand ability of organism to adapt to varied pH levels which can help in developing a coordinated plan for ecosystem management of commercially important species.

Several research studies on corals, crustaceans, algae, molluscs and annelids have pointed out the differences in responses of marine biota to elevated carbon dioxide (Ries *et al.* 2009; Kroeker *et al.* 2010), sea urchins (Dupont *et al.* 2013), echinoderms, coccolithophores, pteropods, foraminifera (Fabry *et al.* 2008), marine

fish (Ishimatsu *et al.* 2008; Munday *et al.*, 2011), and mollusks (Talmage and Gobler 2009).

One important study which indicates that there will not be any impact of ocean acidification on early life stages is that by Munday *et al.* (2011). They found that the larval survival and development including skeletal parts and otolith calcification of the common coral reef fish, the spiny damselfish *Acanthochromis polyanthus* was not affected by high CO₂ levels.

2.7.1 Zooplankton and early life stages

Schindler *et al.* (1985) conducted a unique experiment where they acidified a small lake by changing its original pH from 6.8 to 5. They found that during this period the fishes were not able to accept the low pH. However there were changes in phytoplankton species but the primary production did change and the benthic community also changed completely. Main organisms in the food web like the trout could not survive the stress.

The significance of climate change on changing the zooplankton was understood mostly during end of last decade. The impact of climate change on food web interactions has also been indicated (Hughes, 2000 and Fischlin *et al.*, 2007). Abiotic factors which can vary due to climate change; especially temperature increase and pH variation are expected to play a key role in changing the community structure of zooplankton (Nielsen and Brock, 2009).

Anton Pardo *et al.* (2012) observed that changes in community composition in different ecosystems can lead to indirect effects. It can change the trophic level and when population of small zooplankton like cladocerans become low, it can affect the transparency of the ecosystem. This can lead to more turbid situations which can reduce the growth of macrophytes.

Kawamura *et al.* (2015) conducted experiments to evaluate the effect of pH on post larvae and early juveniles of the freshwater prawn, *Macrobrachium rosenbergii*. In this study, they found that pH variation was not by CO₂. They

observed that the survival, growth, and quality of carapace post larvae and early juveniles were negatively affected by pH 5 and especially pH 4. Ndubuisi *et al.* (2015) observed that reduced pH condition had a negative impact on the growth rate of *Clarias gariepinus* fry and they suggested that the water quality of the farms must be regularly monitored.

Liu *et al.* (2018) have described the advances made by the U.S. National Oceanic and Atmospheric Administration's (NOAA) Coral Reef Watch (CRW) program. It operates a global 4-Month Coral Bleaching Outlook system for shallow water coral reefs in collaboration with NOAA's National Center for Environmental Prediction (NCEP). They have stated that this is the first and only freely available global coral bleaching prediction system, which provides decision support advisories related to coral reefs since 2012.

MATERIALS AND METHODS

CHAPTER 3

MATERIALS AND METHODS

Understanding the need to evaluate the response of selected commercially important species to sudden abiotic stress condition, a series of experiments were conducted. The key abiotic parameters whose stress had to be evaluated were selected and the experimental procedures were charted out. Key resources of various groups like finfishes, shellfishes and plankton were selected and the experimental set up was designed and planned. The criteria for categorization into climate based categories like resilient and vulnerable based on the response to sudden stress and revival were identified. The details of the experiments conducted and the methods by which the data were analyzed during the period October 2017 to July 2018 to meet the objectives mentioned in Chapter 1 are given below.

3.1 Location

The experiments were conducted in the ocean acidification laboratory of Fishery Environment Management Division of ICAR- Central Marine Fisheries Research Institute (ICAR- CMFRI), Kochi Kerala, India.

3.2 Abiotic parameters selected

3.2.1 Salinity

Salinity was selected as a key abiotic parameter which is affected by extreme weather events like high precipitation, storm surges and unusual inundations. These events can either lower the salinity for short periods which range from one day to two days to less than a week. Though in these situations, the extreme events get prolonged, in the experiments, we have considered simulation of short term effects up to 96 hrs. Salinity can increase in coastal ecosystem due to monsoon deficient condition or other effects such as high sea surface temperature (SST) combined with evaporation.

3.2.2 Temperature

Temperature has been identified as one of the major abiotic parameters associated with climate change. In natural habitat this can become higher than normal due to factors like droughts, evaporation or less water flow; which can act either singly or as a combined effect of all the three. There will be other natural phenomenon also like the El Nino which can change the temperature beyond the optimum.

3.2.3 pH

It is well known that the atmospheric carbon dioxide (CO₂) dissolves in ocean and reduces the pH and the process is known as ocean acidification.

3.3 Types of experiment

Three different types of short –term experiments were conducted.

- 1) Response to sudden salinity stress and its revival
- 2) Response to sudden temperature stress and revival
- 3) Impact due to varied water pH

3.3.1 Response to sudden salinity variation and revival.

The selected fauna were subjected to stress salinities 5, 10, 15, 20, 25, 30, 35, 40 and 45 ppt. In each experiment, the ambient salinity varied depending upon the source of the test animal. The lower salinities were prepared by adding freshwater to 35 ppt sea water. The higher salinities were available in the laboratory which was maintained by evaporation earlier.

For each salinity treatment, triplicates were maintained. The ambient salinity of the test animal source was considered as control. Test animals (N-60) were stocked in each replicate for fin fishes, mussels, clams and shrimps. For plankton, the density was different and is described in the respective section. The mortality of the animals were noted at intervals of 6, 12, 24, 36, 48, 72 and 96 hrs. At the end of

each stipulated hour the number of dead animals were noted in each replicate treatment. From the number dead, the percentage mortality during direct stress (**A**) was estimated by

$$\frac{\text{Number of dead animals}}{\text{Total stocked in the beginning}} \times 100$$

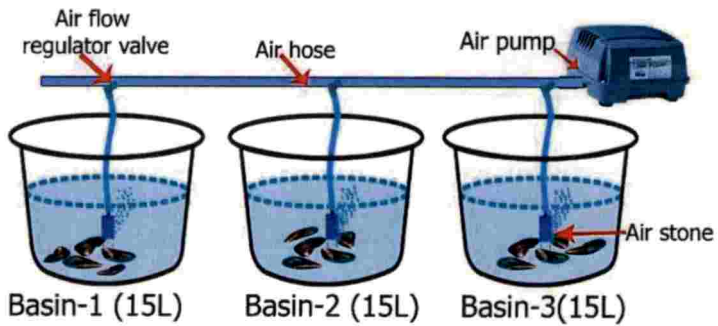
The surviving ones were transferred immediately to their respective ambient salinity and their survival was observed for a period of 24 hrs. This was considered as the revival period. The percentage mortality during the revival (**B**) period was estimated by

$$\frac{\text{Number of dead animals during revival period}}{\text{Total stocked in the stress treatment}} \times 100$$

From these two mortality estimates, the total mortality due to sudden exposure to the particular salinity was estimated.

Layout of Salinity Experiment

Exposure to stress (5 ppt)



Revival from stress (35ppt)



Control without stress (35ppt)

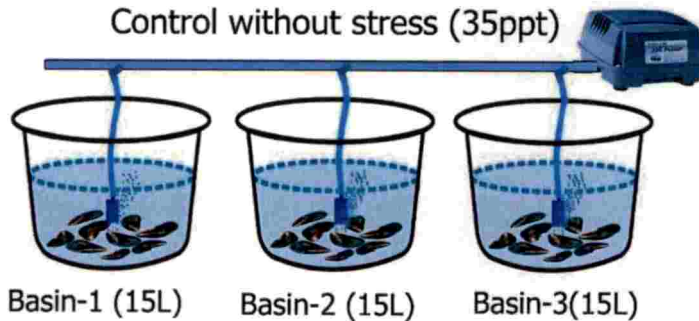


Fig 1: Schematic diagram of the layout of the experimental set up for sudden exposure of mussels to salinity stress followed by revival

3.3.2 Response to sudden temperature variation and revival

Layout of Temperature Experiment

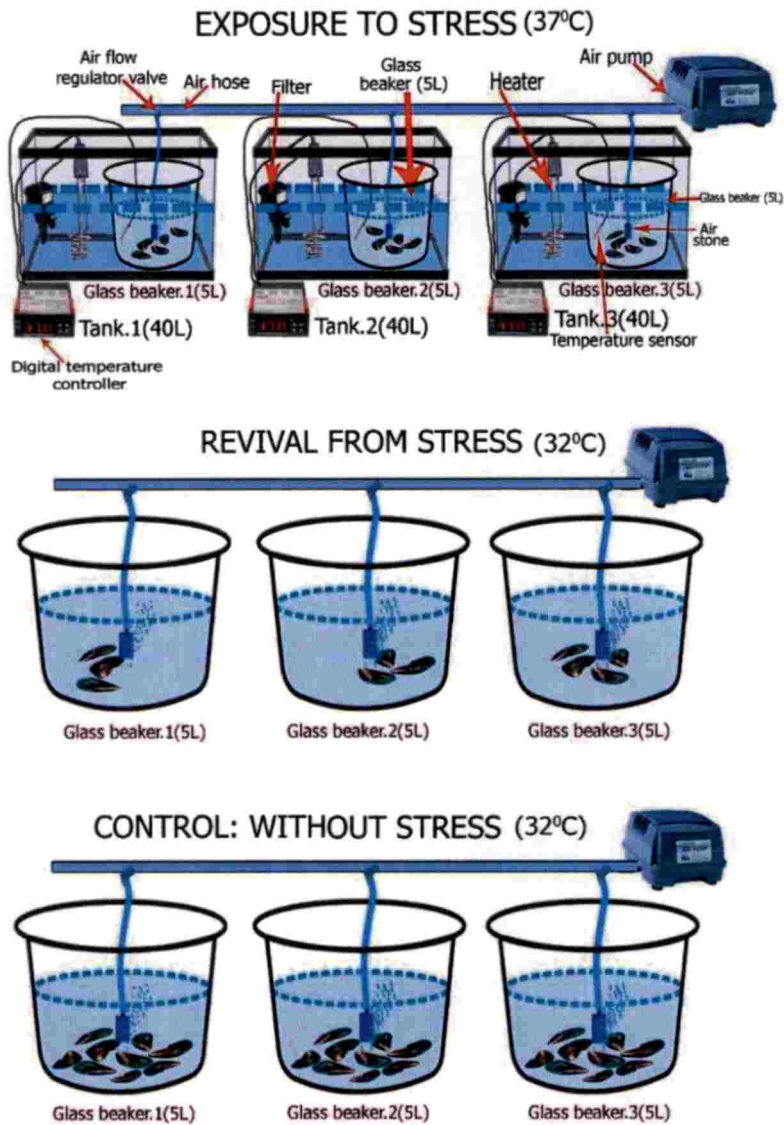


Fig 2: Schematic diagram of the layout of the experimental set up for sudden exposure of mussels to temperature stress followed by revival

In the experiments conducted to evaluate the response to sudden exposure to heat stress, temperature was maintained through thermo-stats. For each test animal, the temperature tested were 30, 32, 34, 36, 38, 40 and 42°C .The temperature

fluctuations were checked by a thermometer. If 100 % mortalities were observed in lower temperatures itself, then higher temperatures treatments were discontinued

The mortalities and survival percentages in each treatment during exposure to stress and revival was calculated as for salinity experiments described in (3.3.1).

3.3.3 Behavioural changes

Apart from mortality any behavioral change by the test animals in different stress conditions were also noted. This was done for salinity and temperature treatments. Byssal thread formation or attachment in mussels. Erratic swimming and other factors related to health were noted.

3.3.4 Climate based categorization

Based on the percentage of total mortality, four categories were identified as given below for each of the treatments for all the species. The four different colour codes were also identified for easy identification.

3.3.5 Climate based categorization

Category	Resilient (R)	Moderately Resilient (MR)	Moderately Vulnerable (MV)	Vulnerable (V)
Mortality percentage	0 - 25	26 - 50	51 - 75	>75

3.3.6 Impact due to varied water pH

The different pH ranges were prepared by passing carbon dioxide through sea water. Prior to start of experiments, a series of standardization procedures were carried out. This was done by passing CO₂ from the cylinder to a known volume of water for a fixed period (seconds).



Plate 1: Carbon dioxide flow regular and recorder

developed by Fishery Environment Management Division of CMFRI was used. The whole unit is controlled by an electrical power supply with human interface device or HID. After immersing the external release probe in to the sea water container, the HID is pressed to activate the device and CO₂ gas is released into the sea water from the cylinder. Input phase consists mainly of a safety regulator cum analogue pressure gauge of external CO₂ cylinder.

Different pH levels were prepared by passing CO₂ for various seconds ranging from 1 to 30. The concentration of CO₂ was estimated by titration method and the pH was read from a digital pH meter (pH tester 10, made by Eutech Instruments) with an accuracy of 0.1.

Based on these repeated trials, the concentration of CO₂ and the pH values were fixed. pH levels of 5.8, 6.2, 6.40, 6.60, 6.70, 6.80, 7.02, 7.2, 7.4, 7.8 and 8.0 were the different treatments.

Prior to exposure to stress pH treatments, the test animals were observed under a microscope and pictures were taken to get a clear idea of the exoskeleton of the zooplankton and the fins of the larvae of clown fish. After exposure also the animals were observed under stereo-zoom microscope (Nikon-SMZ-25, Japan) and their pictures taken by Nikon software (NIS) elements microscope imaging software for comparison and evaluation of the impacts.

3.4 Test animals and experiment procedure

The following marine biota were selected for the study. For salinity and temperature stress simulations and revival experiments the following biota were selected

3.4.1 Finfishes

1. Etroplus suratensis (Pearl spot)

Etroplus suratensis is a very popular Cichlid found in coastal and marine zones in southern India and Sri Lanka. Common names include pearl spot cichlid, banded pearl spot, green chromide and striped chromide. In India, these support a fishery especially in the estuarine regions of Kerala. These are also spread across the country especially in Goa, Tamil Nadu Andhra Pradesh, Orissa and West Bengal. They are omnivorous and feed mainly green algae, detritus, insects, and diatoms. *E. suratensis* is a popular food fish and fetches high prices. In Kerala, it is locally known as 'Karimeen' and is considered a delicacy. In 2010, this species was named the official state fish of Kerala. The following year was proclaimed "The Year of the 'Karimeen'. A pearl spot sanctuary was additionally set up in open Vembanad Lake at Kumarakom, Kerala. These are farmed in earthen ponds and now they are also farmed in simple cages in the back waters.

2. *Trachinotus blochii* (Silver pompano)

Trachinotus blochii (Silver pompano) fishes are good for farming, mainly owing to its fast growth rate, good meat quality, and high market demand. The species is pelagic, very active and is known to be able to acclimatize to lower salinities. This species is suitable for cultivating in the low saline coastal waters as well as in cages. The CMFRI has developed its farming and seed production technologies. This species is promoted as a candidate species for cage farming all along the Indian coast. The response of these species to sudden variation in environmental changes would be helpful to the farmers who invest in this Mariculture program.

3.4.2. Shrimp

1. *Penaeus monodon* (Tiger prawn)

Penaeus monodon, commonly known as the tiger prawn or Asian tiger shrimp, is an economically important species. It contributes significantly to the shrimp fishery and also the aquaculture production. Its natural occurrence is in Indo-Pacific, extending from the eastern bank of Africa and the Arabian Peninsula to the Southeast Asia, the Pacific Ocean, and northern Australia. The species is widely distributed in estuaries and brackish water. The species apparently prefers warm water habitats and live in waters ranging from 28-33°C. It has an estuarine and offshore phase in its life cycle. Adults are found in coastal inshore waters and undergoes breeding or migration to offshore. The eggs, larvae and post-larvae have pelagic existence. The post larvae migrate to the estuary, grow there to juveniles or sub-adults and migrate back into the sea. Juveniles, sub adults and adults are benthic in nature. Tiger shrimp is the second-most widely cultured shrimp species in the world, after white leg shrimp or *Penaeus vannamei*. This species locally known as 'Kara chemmen' in Malayalam is farmed in the semi intensive ponds across the coastal areas. Though it can tolerate wide range of temperature and salinity fluctuations, its response to short term extreme events

is not known. The shrimp farmers are in general a vulnerable group of farmers because of the high risk of virus attack, to farm stock and subsequent crop loss.

3.4.3. Bivalve

1. *Perna viridis* (Green mussel)

Perna viridis or Asian green mussel locally known as “kallumakkaya” is an economically important mussel belonging to the family Mytilidae. Green mussel is a large (80-100 mm) bivalve, with a smooth, elongate green colour shell. These are found attached to hard substratum in the intertidal to shallow littoral zones of the coastal areas. In India, green mussel is found abundantly in the coastal regions of almost all maritime states. This species is an efficient filter feeder, feeding on phytoplankton, small zooplankton, and other suspended fine organic material. Green mussels occur in environments where, temperatures range from 10-35°C. It is a euryhaline species and able to tolerate both hyper saline conditions (40 ppt) and reduced salinities and the optimal salinity range is 27-33 ppt. Green mussel is a commercially important marine bivalve and has a greater role in meeting the increasing protein demands of the human population. Green mussel is farmed extensively in Kerala especially in north Kerala since 1996. This has provided livelihood for several women self-help groups (SHG) and has led to Women empowerment (Kripa and Surendaran, 2008). This species is also farmed in Karnataka (Sasikumar *et al.*, 2006). In recent years, the farmers were affected by mass mortality of farmed green mussel in north Kerala (Mohammed *et al.*, 2016). Since the species is farmed in the estuarine region, the farmers are more vulnerable to crop loss if the salinity drops due to the extreme event like flood and lack of flow of water when drought occurs.

2. *Villorita cyprinoides* (Black clam)

Villorita cyprinoides (black clam), locally known as karuthakakka is a commercially important clam. This species is found in the backwaters of Kerala, mainly in Vembanad backwaters and contributes to 70 % of clam production of the Country. Clam is a natural bio filter with optimum salinity and temperature of 15 ppt and 23°C. It is harvested extensively from the wild population and this exploitation can become a potential threat to this species. *Villorita cyprinoides*, is an endemic brackish water species that cannot tolerate high salinity. They occur in the salinity range of 3 ppt in August and 16 ppt in May (Laxmilatha *et al.*, 2005). Harvested extensively from the wild, thousands of people in Kerala depend on this species for their livelihood and basic protein supply.

3.4.4. Phytoplankton

1. *Isochrysis galbana*

Isochrysis galbana is a unicellular marine algae. They prefer warm waters. This is a commercially important algae which is cultured as feed for larval stages of bivalves and shrimps in hatcheries. This species is given as the first feed in commercial hatcheries of shrimps. It is also used in fin fish hatcheries. Sometimes this is used along with other algae like *Chaetoceros sp.* and *Nannochloropsis* in hatcheries. In the wild, this species is also a part of the planktonic community structure

For ocean acidification studies the following two species were selected

3.4.5. Juvenile of *Amphiprion percula* (Clown fish)

Amphiprion percula or clownfish (otherwise called the Anemone fish) is a common fish that is found around tropical coral reefs. Clownfish with orange with white markings are common yet they can be found in a wide range of colors and shape. They are generally omnivorous. They feed on an extensive variety of algae

and they lay such a large number of eggs at any given moment. The hatchery technology of this species was developed by CMFRI (Madhu *et al*; 2013) and this is popularized as an income generation programme in coastal village of Ramanad District of Tamil Nadu (Johnson *et al.*, 2016).

3.4.6. Zooplankton *Daphnia salina*

Zooplankton *Daphnia salina* (Cladoceran) *Daphnia* is a cladoceran zooplankton, whose body is encased by an uncalcified shell known as the carapace. It has a double wall, between which is the haemo-lymph. The body length of this cladoceran ranges from less than 0.5 mm to in excess of 6 mm. This species is widely used as a feed in fish hatcheries. The small size, fast growth and ease of population has made these very popular live feed organisms. This resource can be affected by the destruction of carbon dioxide in water.

3.4.7. Response to stress: *Eetroplus suratensis*

The seed of pearl spot for the experiment were sourced from a private fish seed supplier from Puthu Vypin. The seed were transported in aerated containers. These were stocked in 500 L FRP tanks with 10 ppt sea water which was continuously aerated. The FRP tank was also fitted with a recirculation system. They were fed with pellet feed of 5mm dia (@VARNA).and acclimatized for five days before exposing them to salinity and temperature response tests.

The length in centimetres and wet weight in grams of 30 fish seed randomly selected were taken using scale and electronic balance respectively. The fishes for experiments were also checked for any external injuries or diseases. The temperature experiments were conducted in glass aquaria of 40 L capacity and salinity in plastic troughs. For each replicate 60 pearl spot were used. The response of the animals were closely monitored and any dead specimen was immediately removed. These were fed twice daily.

During the experiment the salinity in the experimental tanks were checked once in the morning. Continuous aeration was provided. The water in the tank was

changed every day and replaced with water of same salinity or temperature immediately. Feed waste and fecal matter were siphoned out twice daily. Dead fishes were removed immediately. The behavior of fishes was also noted during the experimental period.

3.4.8. Response to stress: *Trachinotus blochii* (Silver pompano)

The seed of silver pompano was sourced from the hatchery of CMFRI at Mandapam Regional Centre. They were transported in CMFRI vehicle with good aerated packs in cool condition. The fishes were acclimatized to the laboratory condition in 35 ppt salinity in 500 L FRP tank with aeration. These were fed twice daily with pellet feed of 1 to 1.5 mm dia (Varna) at of 8 % of body weight. The feed waste as well as fecal matter were removed daily. Any fish with distorted movements or injury was removed immediately. Thirty fishes were measured. Total length and total weight were recorded as described in section 3.4.7. Sixty fishes were stocked in each replicate of the different salinity and temperature treatment and in the respective control sets. All experimental protocols as described in section 3.4.7 were followed



Plate 2: Recording observation on the fish *T. blochii* for weight (left) and total length (right)



Plate 3: A view of the part of experimental set up for salinity stress experiments

3.4.9. Response to stress: Post larvae (PL 21) *Penaeus monodon* (Tiger prawn)

Post larvae of *P.monodon* were sourced from a private hatchery at Azhikode, Kerala. They were transported in aerated water in thick plastic packs. In the laboratory they were stocked in 10 ppt salinity and maintained in glass aquaria with aeration. They were acclimatized for 5 days. During this period they were fed with larval pellet feed twice daily. Continuous aeration was provided.

In the experimental tanks aeration was provided and all husbandry methods as described in 3.4.7 were followed. In addition to this, the moulting process was also observed.

3.4.10. Response to stress: *Perna viridis* (Green mussel)

Mussel seed and adult mussels were collected from the sub-tidal region of the rocky shore along Njarakkal beach, Ernakulum district, Kerala. These were brought to the laboratory in cool condition by wrapping with a moist cotton cloth. In the laboratory, the mussels were washed gently and all silt were removed. After this, all attached fauna were gently scraped off and the byssus threads were carefully detached to make them free. Following this, they were stocked in ambient sea water of 35 ppt and acclimatized under laboratory condition for five days. During this period they were fed twice daily with 100 ml of pure culture of *Chaetoceros*

calcitrans (Cell density = 3.6×10^5 cells per ml) obtained from the live feed culture unit of Marine Hatchery of CMFRI.



Plate 4: Measurement of green mussel *Perna viridis* using digital vernier calipers.



Plate 5: A view of the live feed culture section of Marine Hatchery of CMFRI at Kochi

For measurement 30 numbers each from the seed and adult stock were randomly selected and measured using a digital vernier calipers (MITOSYO make) and total was taken using a digital balance. The length from tip of umbo to valve

opening was considered as length, width as the maximum measurement along the antero - posterior side and depth as the maximum thickness. Behavioural changes like attachment by formation of byssal threads, movement, fecal formation and spawning were observed in different treatments. The dead animals were removed immediately from the test container.

3.4.11. Response to stress: *Villorita cyprinoids* (Black clam)

The clams required for the study were collected from the Muhamma region of the Vembanad lake of Alappuzha district. The clams were collected from the local fishermen who hand-picked the clams after diving in water. The clams were transported to the laboratory in a bucket containing water collected from the natural bed. A piece of cotton soaked with water was placed in the upper and lower surface of the bucket to reduce stress and injury by mechanical movements during transportation. In the laboratory, they were maintained in plastic troughs with 10 ppt salinity. All husbandry methods and measurements as mentioned in section 3.4.10 were adopted.

3.4.12. Experiment set up in laboratory

The experiments were conducted in triplicates with a controlled salinity of 10 ppt and treatments having salinity range of 5 to 45 ppt. The stocking density was 60 clams per replicate. The observed salinity showed ± 1 standard deviation. The clams were fed once in day with 1000 ml of diatom *Chaetoceros calcitrans*. Juveniles and adults clams were maintained separately. Mortality, filtration rate and water quality parameters such as pH and ammonia were frequently checked daily with standard protocols during stressed and revival after stressed conditions in every salinity.

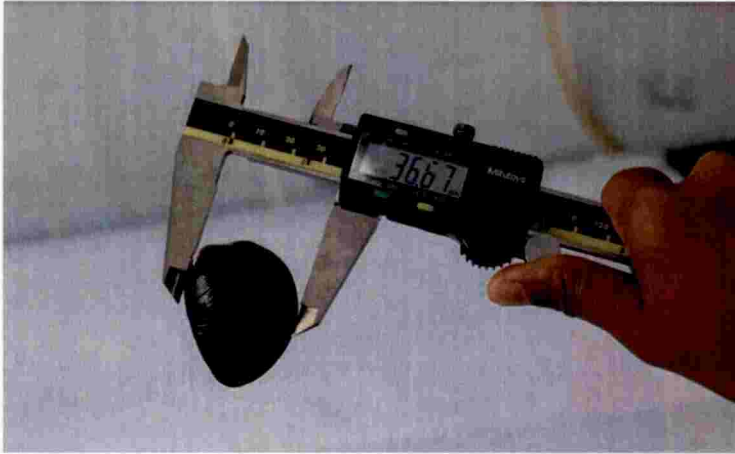


Plate 6: Measuring the black clam *Villorita cyprinoides* with digital vernier calipers

3.4.13. Response to stress by *Isochrysis galbana*

Pure culture of *I. galbana* obtained from live feed section of CMFRI marine hatchery were used as stock culture for large scale culture for the experiments. These live feed culture was developed as per standard protocols using Walne's medium. The cell density was counted in a haemocytometer as described by Gopinathan (1982). After the stock reached the growing stage they were exposed to sudden temperature increase of 32, 34 and 36°C for 6,12, 24, 36, 48, 56,72 and 96 hours respectively. The treatments were in triplicate and the cell densities per ml was estimated at the end of each treatment. The revival was in 24°C for each of the stress treatments. The control was maintained at 24°C throughout the experiment in the algal culture section of the laboratory of FEMD, CMFRI.



Plate 7: Experimental set up in algal culture lab of FEM Division (left); Haufkins flask with stock culture of *I.galbana* (right)

3.4.14. Response to different pH by juvenile clown fish, *Amphiprion percula*

Juvenile clown fish produced in Marine Hatchery of CMFRI were used for the experiment. The larvae were maintained in the laboratory for five days with continuous aeration and feed. The water was changed daily and the water quality during this period was checked by testing the dissolved oxygen levels and pH.

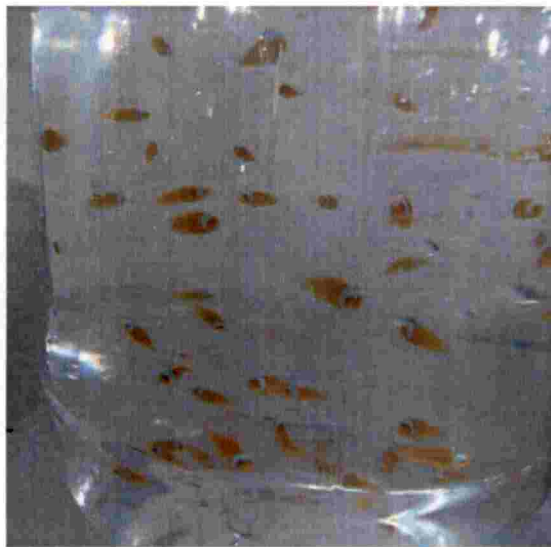


Plate 8: A view of the juvenile clown fish *Amphiprion percula* being acclimatized in the FEMD ocean acidification laboratory of CMFRI

3.4.15. Response to different pH by *Daphnia salina* (Cladoceran)

Daphnia salina cultures in the live feed section of CMFRI marine hatchery were used for the experiments. These were stocked in five litre beakers with good aeration. Algal feed was given to them twice daily. These were examined under the stereo zoom microscope and images taken.

From this stock, 50 healthy animals were selected and exposed to water with specific pH and carbon dioxide estimated. The movements were observed and at the end of 8 hrs, their mortality was noted and live one taken for detailed observation under microscope. The images were captured under the stereo- zoom microscope using the image analysis software. These were preserved in buffered formalin for further analysis if required.



Plate 9: Zooplankton culture maintained in marine hatchery of CMFRI



Plate 10: A view of *Isochrysis galbana* in a hemocytometer as observed under microscope (Magnification: 20X)

3.5 Analytical methods followed

3.5.1 Dissolved oxygen

Dissolved oxygen was estimated by modified Winkler method (Grasshoff, 1983). The water samples collected for analysing dissolved oxygen were fixed immediately with manganese sulphate (Winkler - A) solution and alkali - iodide reagent (Winkler - B). Dissolved oxygen was estimated on the same day by adding concentrated H_2SO_4 . The liberated iodine from potassium iodide in acid medium was titrated against standard sodium thiosulphate solution using starch as indicator. The amount of oxygen in the sample was expressed in $mg L^{-1}$.

3.5.2 Total Ammonia – N

Total ammonia - N was estimated by the indo-phenol method described by Solorzano, (1969).

3.5.3 Carbon dioxide

Carbon dioxide in water was estimated by titration method described by Milburn *et al.* (1960).

3.5.4 Salinity

Salinity of the water was measured using a refractometer.

3.5.5 Temperature

Temperature of water samples were measured immediately after collection by using a digital thermometer (OAKTON, USA) with accuracy $\pm 0.1^{\circ}\text{C}$.

3.6 Statistical analysis

The statistical analysis of the data was carried out using the software IBM SPSS Statistics 20. Log- 10 transformed percentage survival rate was used to analyze the variance (ANOVA) of each species with different salinities and temperature. Pearson correlation analysis in Microsoft Excel was used to find the significance relationship between the varying salinities and survival rate of each species

RESULTS

CHAPTER 4

RESULTS

4.1 Juvenile *Etroplus suratensis* (pearl spot)

Juveniles of *E. suratensis* of average length $5.53 \text{ cm} \pm 0.27 \text{ SD}$ and average total weight $3.5 \text{ gm} \pm 0.29 \text{ SD}$ were used for the experiments. The dissolved oxygen level was 7.4 mg L^{-1} . The control salinity was 10 ppt, ambient temperature was 30°C and pH was 7.26 throughout the experimental period.

4.1.1 Response to salinity variation

Juvenile *E. suratensis* was found to be very sturdy and survived in sudden exposure to salinities from 0 to 36 ppt for upto 96 hours indicating that there can be extreme events like floods where fresh water ingression can lower salinities suddenly. Similarly, their survival in higher salinity of 40 ppt was 100 % upto 12 hours, but in 24 hours mortality was $58.9 \% \pm 9.7 \text{ SD}$ of which $7.8\% \pm 4.2 \text{ SD}$ was during the revival period (Table 1). However, they could not tolerate this salinity for 36 hours and there was complete mortality during this period. The mortality was very fast in 45 ppt and in 6hr there was $65 \% \pm 8.9 \text{ SD}$ mortality. Within 12 hr all juveniles exposed to this salinity suffered mortality (Table 2).

The experiment on juvenile *E. suratensis* for testing the survival during sudden variation in salinity indicates that it is a sturdy species and can survive sudden salinity variations in the range 0 to 36 ppt without any mortality, indicating its high resilience capacity from extreme events which lower the salinity. The juvenile *E. suratensis* was vulnerable to sudden high salinities above 36 ppt.

The study indicates that farmers should avoid stocking during summer months. If sudden salinity increase is expected then postpone stocking till salinity becomes less than 36 ppt. This also indicated that juveniles of *E. suratensis* are vulnerable to drought like situations. Hence it is better to plant more shade giving plants especially mangroves which can protect natural resources during peak summer period.

Table 1: Mean mortality of juvenile pearl spot (*Etroplus suratensis*) on sudden exposure to 40 ppt salinity and subsequent revival in ambient salinity (10 ppt)

Time (hrs)	Mortality during exposure in 40 ppt (N = number,60)		Mortality during revival in ambient salinity (10 ppt)		Mortality in 40 ppt treatment during exposure and revival period	
	N	%	N	%	N	%
24	30.7 ± 3.3	51.1 ± 5.5	4.7 ± 2.5	7.8 ± 4.2	35.3 ± 5.8	58.9 ± 9.7
36	56.7 ± 2.9	94.4 ± 4.8	3.3 ± 2.9	5.6 ± 4.8	60	100

Table 2: Mean mortality of juvenile pearl spot (*Etroplus suratensis*) on sudden exposure to 45 ppt salinity and subsequent revival in ambient salinity (10 ppt)

Time (hrs)	Mortality during exposure in 45 ppt (N = number,60)		Mean mortality during revival in ambient salinity (10 ppt)		Mortality in 45 ppt treatment during exposure and revival period	
	N	%	N	%	N	%
6	36.3 ± 5.8	60.6 ± 9.6	2.7 ± 1.7	4.4 ± 2.8	39 ± 5.4	65 ± 8.9
12	53.6 ± 2.1	89.4 ± 3.4	6.3 ± 2.1	10.3 ± 3.7	60	100

Table 3: Analysis of variances with salinity, time interactions and survival rate of *Etroplus suratensis*

Source		Type I SS	df	Mean Square	F	Sig.
Salinity	Hypothesis	25.28	2	12.64	5.648	0.049*
	Error	11.494	5.136	2.238a		
Time	Hypothesis	9.467	3	3.156	2.39	0.24 NS
	Error	3.96	3	1.320b		
Salinity * Time	Hypothesis	3.96	3	1.32	289.011	0.00**
	Error	00.73	16	00.5c		

*Significant NS: Not significant

In general, the survival rate of *E. suratensis* was significant is ($P < 0.05$) at 40 and 45 ppt. The survival rate with time was not significant and the salinity-time interaction was highly significant ($P < 0.01$) with the percentage survival rate.

4.1.2 Response to temperature variation

Juvenile *E. suratensis* survived in all temperatures from 32 to 40°C. In 42°C the mortality progressively increased in 6, 12, 24 and 36 hours with values 26.7 ± 7.2 SD, 41.2 ± 6.7 SD, 88.3 ± 5.9 SD and $90.5 \% \pm 7.5$ SD respectively. At 48 hours, there was 100 % mortality. The mortality during the revival period ranged between $1.7\% \pm 2.4$ SD in 6 hours to $12.2 \% \pm 3.4$ SD in 36 hours (Table 4). The stress tests have indicated that juveniles of pearl spot are resilient to sudden shocks of temperature up to 10°C increase in temperature from 30°C (Table 5). But they become vulnerable after 40°C and cannot survive in this temperature beyond 12 hrs. There was no mortality during the revival period of 12 hours exposure. So at this period it moderately resilient and farmers can act on this and save their stock by timely action.

These results indicate that in the natural bed shallow areas which were prone to high water temperature must be developed with adequate shades by planting mangroves. Or if water flow is reduced, attempts should be made to restore water flow so that temperature is dissipated. Farmers should take care to reduce the heat by better water circulation and shades. One observation on general health was the skin infection in test animals after during the recovery phase of the experiment. When the juveniles are exposed to extreme temperature condition and back to the ambient condition they were affected by a bacterial disease (plate 11), known as fin rot, and 100 % mortality was recorded after 48 hours.

Table 4: Mean mortality of juvenile pearl spot (*Etroplus suratensis*) on sudden exposure to 42°C temperature and subsequent revival in ambient temperature 30°C.

Time (hrs)	Mortality during exposure in 42° C (number, n =60)		Mortality during revival in ambient temperature (30°C)		Mortality in 42°C treatment during exposure and revival period	
	N	%	N	%	N	%
6	14 ± 4.3	23.3 ± 7.2	1 ± 1.4	1.7 ± 2.4	16 ± 4.3	26.7 ± 7.2
12	20.3 ± 2.9	33.9 ± 4.8	4.3 ± 2.1	7.2 ± 3.4	24.7 ± 4	41.2 ± 6.7
24	49 ± 4.5	81.7 ± 7.6	4 ± 1.4	6.7 ± 2.4	53 ± 3.6	88.3 ± 5.9
36	47 ± 2.4	78.3 ± 4.1	7.3 ± 2.1	12.2 ± 3.4	54.3 ± 4.5	90.5 ± 7.5
48	56.7 ± 2.5	94.4 ± 4.2	3.3 ± 2.5	5.6 ± 4.2	60	100

Table 5: Analysis of variances with temperature, time interactions and survival rate of *Etroplus suratensis*

Source		Type I SS	df	Mean Square	F	Sig.
Salinity	Hypothesis	83.44	2	41.72	29.55	0.00**
	Error	12.52	8.87	1.412a		
Time	Hypothesis	10.13	6	1.69	1.73	0.34 NS
	Error	3.11	3.19	0.974b		
Temperature * Time	Hypothesis	3.46	4	0.87	1.94	0.13 NS
	Error	13.37	0.3C	0.446c		

***Significant NS: Not significant**

The survival rate of *E. suratensis* in 40 and 42°C was found to be highly significant ($P < 0.01$). The percentage survival between time and temperatures – time interaction was not significant.

68



Plate 11: Fin rot disease in juvenile of *E. suratensis* which was exposed to sudden temperature increase from 30°C to 42°C.

4.2 Juvenile *Trachinotus blochii* (Silver pompano)

Juveniles of *T. blochii* of average length 5.0 cm \pm 1.9 SD and average total weight 2.65 gm \pm 0.32 SD were used for the experiments. The dissolved oxygen level was 6 mg L⁻¹. The control salinity was 35 ppt, ambient temperature was 30°C and pH was 7.6 throughout the experimental period.

4.2.1 Response to salinity variation

The juveniles of *T. blochii* showed 100 % survival in 25 to 35 ppt salinities up to 96 hours. These young fishes were found to be vulnerable to sudden lowering of salinity. Though they survived upto 36 hours in 5 ppt, the mortality was high 43.3 % \pm 5.4 SD, 76.1 % \pm 7.5 SD and 87.2 % \pm 6.7 SD in 12, 24, and 36 hours (Table 6). This indicated that action must be taken before 12 hours to remove the stock from low salinity stress. In 10, 15 and 20 ppt also high mortality was observed after 12 hours indicating the vulnerability of the juvenile *T. blochii* to sudden exposure of low salinity (Tables 7, 8 & 9). Within 36 hours there was complete mortality. The species was so stressed that there was no survival after 76 hours. But within 6 hours there was no mortality either during exposure or also during revival. Hence this information can increase the preparedness of the farmers for saving the stock. In 20

ppt, the survival was low and the juvenile could not survive beyond 48 hours. This shows that even sudden short spells of extreme precipitation or other events which can lower the salinity is detrimental to *T. blochii* juvenile fishes.

The juveniles of *T. blochii* were not tolerant to higher salinity like 40 and 45 ppt. The percentage of total mortality was low ($17.8 \% \pm 4.2$ SD) in 6 hours at 40 ppt salinity but increased to $44.4 \% \pm 8.3$ SD in 12 hours and was more than 50 % in 24 hours (Table 10). In 45 ppt there was complete mortality in 12 hours (Table 11). These results clearly indicated that sudden exposure to high salinity is also fatal to the juvenile species of *T. blochii*. Hence action should be taken to shift the juveniles to lower salinities in case of extreme events which can increase the salinity of the area. In natural stocks, these species cannot survive in sudden shifts in temperature and salinity extremes.

Behavioural changes: On exposure to stress salinity of 5, 10 and 45 ppt, the juvenile fishes were found to swim fast. Their swimming was erratic and they did not accept feed as inferred from the unused feed. In other salinities, movement was normal and they accepted feed.

Climate related resilience: The experimental results indicated that juveniles of *T. blochii* grown in 30 ppt salinity can be resilient to 5 ppt variation (at 25 and 35 ppt) as sudden shocks. Beyond that they are vulnerable to salinity variation. At 20 and 40 ppt they are moderately vulnerable. But are vulnerable in low salinities and high salinities. Hence farmers have to be cautious and must monitor salinity variations during extreme events and take precautions to prevent mass mortalities.

Table 6: Mean mortality of juvenile pompano (*Trachinotus blochii*) on sudden exposure to 5 ppt salinity and subsequent revival in ambient salinity (30 ppt)

Time (hrs)	Mortality during exposure in 5 ppt (N = number,60)		Mortality during revival in ambient salinity (30 ppt)		Mortality in 5 ppt treatment during exposure and revival period	
	N	%	N	%	N	%
12	26 ± 3.3	43.3 ± 5.4	0	0	26 ± 3.3	43.3 ± 5.4
24	45.7 ± 4.5	76.1 ± 7.5	0	0	45.7 ± 4.5	76.1 ± 7.5
36	52.3 ± 4	87.2 ± 6.7	0	0	52.3 ± 4	87.1 ± 6.7
48	60	100	0	0	60	100

Table 7: Mean mortality of juvenile pompano (*Trachinotus blochii*) on sudden exposure to 10 ppt salinity and subsequent revival in ambient salinity (30 ppt).

Time (hrs)	Mortality during exposure in 10 ppt (N = number,60)		Mortality during revival in ambient salinity (30 ppt)		Mortality in 10 ppt treatment during exposure and revival period	
	N	%	N	%	N	%
12	32.7 ± 4.1	54.4 ± 6.8	0	0	32.7 ± 4.1	54.5 ± 6.8
24	45.7 ± 4.5	76.1 ± 7.5	0	0	45.7 ± 4.5	76.1 ± 7.5
36	46.3 ± 4.6	77.2 ± 7.7	0	0	46.3 ± 4.6	77.1 ± 7.7
48	48 ± 1.6	80 ± 2.7	0	0	48 ± 1.6	80 ± 2.7
72	60	100	0	0	60	100

Table 8: Mean mortality of juvenile pompano (*Trachinotus blochii*) on sudden exposure to 15 ppt salinity and subsequent revival in ambient salinity (30 ppt)

Time (hrs)	Mortality during exposure in 15 ppt (N = number,60)		Mortality during revival in ambient salinity (30 ppt)		Mortality in 15 ppt treatment during exposure and revival period	
	N	%	N	%	N	%
12	44.7 ± 4.5	74.4 ± 7.5	1.3 ± 1.9	2.2 ± 3.2	46 ± 5	76.6 ± 8.3
24	52.3 ± 3.7	87.2 ± 6.1	0	0	52.3 ± 3.7	87.1 ± 6.1
36	52.3 ± 3.4	87.2 ± 5.7	0	0	52.3 ± 3.4	87.1 ± 5.7
48	60	100	0	0	60	100

Table 10: Mean mortality of juvenile pompano (*Trachinotus blochii*) on sudden exposure to 45 ppt salinity and subsequent revival in ambient salinity (30 ppt)

Time (hrs)	Mortality during exposure in 45 ppt (N = number,60)		Mortality during revival in ambient salinity (30 ppt)		Mortality in 45 ppt treatment during exposure and revival period	
	N	%	N	%	N	%
6	54 ± 2.4	90 ± 4.1	1 ± 0.8	1.7 ± 1.3	55 ± 1.6	91.7 ± 2.7
12	60	100	0	0	60	100

Table 9: Mean mortality of juvenile pompano (*Trachinotus blochii*) on sudden exposure to 40ppt salinity and subsequent revival in ambient salinity (30 ppt)

Time (hrs)	Mortality during exposure in 40 ppt (N = 60)		Mortality during revival in ambient salinity (30 ppt)		Mortality in 40 ppt treatment during exposure and revival period	
	N	%	N	%	N	%
6	10.7 ± 2.5	17.8 ± 4.2	0	0	10.7 ± 2.5	17.8 ± 4.2
12	26.7 ± 5	44.4 ± 8.3	0	0	26.7 ± 5	44.8 ± 8.3
24	33 ± 3.3	55 ± 5.5	0	0	33 ± 3.3	55 ± 5.5
36	53 ± 4.1	88.3 ± 6.8	0	0	53 ± 4.1	88.3 ± 6.8
48	60	100	0	0	60	100

Table 11: Analysis of variances with salinity, time, interactions and survival rate of *Trachinotus blochii*

Source		Type I SS	df	Mean Square	F	Sig.
Salinity	Hypothesis	112.89	6	18.82	14.06	0.00**
	Error	9.35	6.99	1.338a		
Time	Hypothesis	24.03	4	6.01	14.85	0.00**
	Error	8.09	2.0	0.404b		
Salinity * Time	Hypothesis	8.09	2.0	0.4	13.98	0.00**
	Error	1.74	6.0	0.0290		

**** Significant**

The percentage survival rate at 5, 10, 15, 20, 40 and 45 ppt is ($P < 0.01$) which shows high significance with time, salinity and salinity-time interaction.

4.2.2 Response to temperature variation

Survival was high, up to 100 % when the juveniles reared in 30°C were exposed to 32 and 34°C. Survival of juvenile *T. blochii* was low in higher temperature also. It was observed that these were 35 % ± 8 SD mortality at 36°C of which 31.7 % ± 11 SD was during the revival period. Though the mortality during the exposure period of 36°C was low (3.3 % ± 3 SD), these juveniles couldn't survive

the stress and there was no mortality during the revival period. In 24 hours, these was $53.3 \% \pm 6$ SD mortality and in 36 hours there were complete mortality. At 38°C , the juvenile species suffered $81.1 \% \pm 7$ SD mortality during 12 hour *T. blochii* exposure and 100 % mortality in 24 hours. The experiment shows that juvenile *T. blochii* cannot tolerate higher salinities beyond 6 hours.

Climate related resilience: Juvenile *T. blochii* were resilient to sudden increase in temperature up to 4°C , but beyond this they were vulnerable.

Behavioural changes: On exposure high temperatures of 37 and 38°C , the juvenile fishes were found to swim fast. Their swimming was erratic and they did not accept feed. In other temperatures, movement was normal and they accepted feed.

Table 12: Mean mortality of juvenile pompano (*Trachinotus blochii*) on sudden exposure to 36°C temperature and subsequent revival in ambient temperature (32°C)

Time (hrs)	Mortality during exposure in 36°C (number, n =60)		Mortality during revival in ambient temperature (32°C)		Mortality in 36°C treatment during exposure and revival period	
	N	%	N	%	N	%
12	2 ± 2	3.3 ± 3	19 ± 6	31.7 ± 11	21 ± 5	35 ± 8
24	32 ± 4	53.3 ± 6	0	0	32 ± 4	53.3 ± 6
36	58.7 ± 2	97.8 ± 3	1.3 ± 2	2.2 ± 3	60	100

Table 13: Mean mortality of juvenile pompano (*Trachinotus bolchii*) on sudden exposure to 38°C temperature and subsequent revival in ambient temperature (32°C)

Time (hrs)	Mortality during exposure in 38°C (number, n =60)		Mortality during revival in ambient temperature (32°C)		Mortality in 38°C treatment during exposure and revival period	
	N	%	N	%	N	%
12	48.7 ± 4	81.1 ± 7	0	0	48.7 ± 4	81.1 ± 7
24	60	100	0	0	60	100

Table 14: Analysis of variances with temperature, time, interactions and survival rate of *Trachinotus blochii*

Source		Type I SS	df	Mean Square	F	Sig.
Temperature	Hypothesis	39.83	2	19.92	8.41	0.019*
	Error	14.93	6.3	2.368a		
Time	Hypothesis	14.38	4	3.6	3.16	0.15 Ns
	Error	4.56	4	1.140b		
Temperature X Time	Hypothesis	4.56	4	1.14	0	0.00**
	Error	0.11	20	0.06c		

**** Significant NS: Not significant**

The percentage survival rates of juvenile *T. blochii* at temperature 36° and 38°C (Table 14 and Fig. 6) are not statistically significant, temperature ($P < 0.05$) and temperature-time interaction are highly significant ($P < 0.01$)

4.3 PL -21 *Penaeus monodon* (Tiger prawn)

Post larvae of average length 1.5 cm \pm 0.37 SD were used for the experiments. The dissolved oxygen level was 6.5 mg L⁻¹. The control salinity was 10 ppt, ambient temperature was 30°C and pH was 7.06 throughout the experimental period.

4.3.1 Response to salinity variation

The post larvae of tiger prawn *Penaeus monodon* had high tolerance to salinity variation. There was 100 % survival when the larvae were exposed to a lower salinity of 5 ppt and higher salinities of 15, 20, 25, 30 and 35 ppt salinities up to 96 hour. The post larvae of *P.monodon* (Pl -21) was able to survive in all lower salinities and also up to 35 ppt. Sudden exposure and revival in 40 ppt was found to be tolerable up to 72 hour. Subsequently in 96 hours there was high mortality of 97.8 % \pm 2.1 SD (Table 15). At a salinity of 45 ppt, the mortality was high even within 6 hours and there was 100 % mortality in 12 hours (Table 16). This indicates that the post larvae of *P.monodon* cannot tolerate in higher salinities.

Behavioural response: On exposure to 10 and 35 ppt salinities within 6 hours, the post larvae were found to moult. In 45 ppt, the feed acceptance was low. In the revival phase they were found to be more active.

Climate related resilience: The post larvae of *P.monodon* were found to be resilient to low salinities and even salinities up to 40 ppt on sudden exposure from 10 ppt. Thus even 25 ppt increase in salinity and 5ppt lowering was tolerable, indicating high resilience to salinity variation. However, hyper saline condition, in which salinity was above 40 ppt made these larvae vulnerable.

Table 15: Mean mortality of PL- 21 tiger prawn (*Penaeus monodon*) on sudden exposure to 40 ppt salinity and subsequent revival in ambient salinity (10 ppt).

Time (hrs)	Mortality during exposure in 40 ppt (N = number,60)		Mortality during revival in ambient salinity (10 ppt)		Mortality in 40 ppt treatment during exposure and revival period	
	N	%	N	%	N	%
96	58.7 ± 1.2	97.8 ± 2.1	1.3 ± 1.2	2.2 ± 2.1	60	100

Table 16: Mean mortality of PL- 21 tiger prawn (*Penaeus monodon*) on sudden exposure to 45 ppt salinity and subsequent revival in ambient salinity (10 ppt)

Time (hrs)	Mortality during exposure in 45 ppt (N = number,60)		Mortality during revival in ambient salinity (10 ppt)		Mortality in 45 ppt treatment during exposure and revival period	
	N	%	N	%	N	%
6	53.3 ± 4.5	88.9 ± 7.5	1.3 ± 0.5	2.2 ± 0.8	54.7 ± 4.7	91.1 ± 7.9
12	60	100	0	0	60	100

Table 17: Analysis of variances with salinity, time, interactions and survival rate of *Penaeus monodon*

Source		Type I SS	df	Mean Square	F	Sig.
Salinity	Hypothesis	38.76	2	19.38	14.02	0.00**
	Error	10.75	7.78	1.382a		
Time	Hypothesis	6.46	4	1.62	1.41	°0.37 Ns
	Error	4.59	4	1.148b		
Salinity X Time	Hypothesis	4.59	4	1.15	56.26	0.00**
	Error	10.41	20	.002		

**** Significant NS: Not significant**

The percentage survival rate of PL-21 *P.monodon* at 40 and 45 ppt within salinity and salinity – time interaction (Table 17) are highly significant ($P < 0.01$).

4.3.2 Response to temperature variation

The post larvae of *P.monodon* could survive sudden exposure to increased temperature up to 36°C. At 38°C, mortality was low (16.6 % ± 7 SD) during the first 6 hours (Table 18). Of this 5 % ± 2.1 SD mortality was during the revival period indicating that the PL -21 could not withstand the stress even for 6 hours. By 12 hours there was 49.5 % ± 7 SD mortality and in 24 hours it rose to 88.3 % ± 6.8 SD. 100 % mortality was observed with 48 hours of exposure to 38°C. This indicates that zooplankton mero plankton group consisting of larvae of shrimps may be vulnerable to higher temperatures.

Climate related resilience: The study indicated that larvae of *P.monodon* were resilient to temperature increase of 6°C from 30 ppt. However, they were vulnerable to higher temperature increase of above 38°C. This information suggests that drought like situations are fatal to shrimp larvae.

Table 18: Mean mortality of PL- 21 tiger prawn (*Penaeus monodon*) on sudden exposure to 38°C temperature and subsequent revival in ambient temperature (30°C)

Time (hrs)	Mortality during exposure in 38°C (number, n =60)		Mortality during revival in ambient temperature (30°C)		Mortality in 36°C treatment during exposure and revival period	
	N	%	N	%	N	%
6	7 ± 4.9	11.7 ± 8.2	3 ± 1.2	5 ± 2.1	10 ± 4.2	16.7 ± 7
12	24 ± 4.9	40 ± 8.2	5.7 ± 1.2	9.5 ± 2.1	29.7 ± 4.2	49.5 ± 7
24	49.3 ± 4.	82.2 ± 6.9	3.7 ± 0.5	6.1 ± 0.8	53 ± 4.1	88.3 ± 6.8
36	55 ± 3.7	91.6 ± 6.2	2.6 ± 1.9	4 ± 2.8	57.6 ± 2.1	95.6 ± 3.4
48	60	100	0	0	60	100

Table 19: Analysis of variance with temperature, time interactions and survival rate of *Penaeus monodon*

Source		Type I SS	df	Mean Square	F	Sig.
Temperature	Hypothesis	94.98	2	47.49	46.64	0.00**
	Error	12.22	12	1.018a		
Time	Hypothesis	6.11	6	1.02	1	0.5 NS
	Error	6.11	6	1.018b		
Temperature X Time	Hypothesis	6.11	6	1.02	37.58	0.00**
	Error	0.76	28	0.27c		

**** Significant NS: Not significant**

The percentage survival rate of *P. monodon* with temperature and temperature- time interactions ($P < 0.01$) was highly significant.

4.4 Juvenile *Perna viridis* (Green mussel)

Juveniles of *P. viridis* of average length 23.65 mm ± 1.33 SD, width 22.84 mm ± 1.35 SD and thickness 18.75 mm ± 1.33 SD with average total weight 12.5gm ± 0.21 were used for the experiments. The dissolved oxygen level was 7.2 mg L⁻¹.

The control salinity was 10 ppt, ambient temperature was 30°C and pH was 7.52 and concentration of ammonia was 0.56 µmol/L, throughout the experimental period.

4.4.1 Response to salinity variation

Juvenile *P. viridis* suffered 100 % mortality in 6 hours of exposure to 5 ppt and could not tolerate 10 ppt salinity also for more than 12 hours (Tables 20 and 21). In 15 ppt salinity there was 88.8 % ± 9 SD mortality in 12 hours of which 12.2 % ± 3.2 SD occurred during the revival period (Table 22). In this salinity they could not survive for 24 hours. The juvenile *P. viridis* could survive for slightly longer period in 20 ppt (Table 23). There was 51.7 % ± 7.6 SD mortality in 6 hours and 11.1 % ± 5.1 SD mortality during the revival period. So total mortality during first 6 hours in 20 ppt was 62.8 % ± 4.2 SD. In 12 hours 74.4 % ± 4.8 SD mortality and in 24 hours it was 87.8 % ± 9.5 SD mortality. All the mussels suffered mortality in 36 hours. This indicated that juvenile *P. viridis* cannot tolerate lower salinities. The juvenile *P. viridis* could not tolerate salinity between 5 to 15 ppt. In 40 ppt there was 100 % mortality in 12 hours while in 45 ppt there was 100 % mortality in 6 hours (Tables 24 and 25). This indicated that seed mussels cannot tolerate higher salinities and lower salinities.

Behavioural response. On sudden exposure to 5 and 10 ppt the *P. viridis* did not secrete any byssal threads and the seed suffered mortality within 2 hrs. In 15 and 20 ppt very few (less than 10%) formed byssal threads but they were not active. In 25 to 35ppt salinities, they secreted byssal threads and were found to be active. They accepted the feed and fecal matter was also observed. They were found to move their foot actively and even rise to the top of the experimental container.

Climate related resilience: The seed mussels were found to have resilience to lower salinity values up to 25 ppt. ie, capacity to withstand 10 ppt, decline from 35 ppt. They were vulnerable to 5 ppt higher range from 35 ppt. They were vulnerable to low salinities below 25 ppt and higher salinity of 40 and 45 ppt. This indicated that high rainfall can be detrimental to mussel farmers. It is suggested that mussel seeds are stocked when the salinity stabilizes at 25 ppt. Similarly high salinity stress can also lead to mortality of stock.

Table 20: Mean mortality of juvenile green mussel (*Perna viridis*) on sudden exposure to 5 ppt salinity and subsequent revival in ambient salinity (35 ppt).

Time (hrs)	Mortality during exposure in 5ppt (N = number,60)		Mortality during revival in ambient salinity (35 ppt)		Mortality in 5 ppt treatment during exposure and revival period	
	N	%	N	%	N	%
6	60	100	0	0	60	100

Table 21: Mean mortality of juvenile green mussel (*Perna viridis*) on sudden exposure to 10 ppt salinity and subsequent revival in ambient salinity (35 ppt).

Time (hrs)	Mortality during exposure in 10 ppt (N = number,60)		Mortality during revival in ambient salinity (35 ppt)		Mortality in 10 ppt treatment during exposure and revival period	
	N	%	N	%	N	%
6	5 ± 3.6	8.3 ± 6	2.7 ± 3.1	4.4 ± 5.2	11 ± 6.2	18.3 ± 10.3
12	39 ± 7	50 ± 11.6	3.7 ± 2.5	6.4 ± 4.1	50.3 ± 6.8	78.3 ± 11.4
24	60	100	0	0	0	100

Table 22: Mean mortality of juvenile green mussel (*Perna viridis*) on sudden exposure to 15 ppt salinity and subsequent revival in ambient salinity (35 ppt)

Time (hrs)	Mortality during exposure in 15 ppt (N = number,60)		Mortality during revival in ambient salinity (35 ppt)		Mortality in 15 ppt treatment during exposure and revival period	
	N	%	N	%	N	%
6	29 ± 2.9	48.3 ± 4.9	2.7 ± 0.9	4.5 ± 1.6	31.7 ± 2.1	52.8 ± 3.4
12	46 ± 5.4	76.7 ± 8.9	7.3 ± 1.9	12.2 ± 3.2	53.3 ± 5.4	88.8 ± 9
24	60	100	0	0	60	100

Table 23: Mean mortality of juvenile green mussel (*Perna viridis*) on sudden exposure to 20 ppt salinity and subsequent revival in ambient salinity (35 ppt)

Time (hrs)	Mortality during exposure in 20 ppt (N = number,60)		Mortality during revival in ambient salinity (35 ppt)		Mortality in 20 ppt treatment during exposure and revival period	
	N	%	N	%	N	%
6	31 ± 4.5	51.7 ± 7.6	6.7 ± 3.1	11.1 ± 5.1	37.7 ± 2.5	62.8 ± 4.2
12	42 ± 4.3	70 ± 7.2	3.7 ± 1.7	6.1 ± 2.8	45.7 ± 3.7	76.1 ± 6.1
24	44.7 ± 4.5	74.4 ± 7.5	8 ± 1.4	13.3 ± 2.4	52.7 ± 5.7	87.8 ± 9.5
36	60	100	0	0	60	100

Table 24: Mean mortality of juvenile green mussel (*Perna viridis*) on sudden exposure to 40 ppt salinity and subsequent revival in ambient salinity (35 ppt)

Time (hrs)	Mean mortality during exposure in 40 ppt (N = number,60)		Mean mortality during revival in ambient salinity (35 ppt)		Mean mortality in 40 ppt treatment during exposure and revival period	
	N	%	N	%	N	%
24	60	100	0	0	60	100

Table 25: Mean mortality of juvenile green mussel (*Perna viridis*) on sudden exposure to 45 ppt salinity and subsequent revival in ambient salinity (35 ppt).

Time (hrs)	Mean mortality during exposure in 45 ppt (N = number,60)		Mean mortality during revival in ambient salinity (35 ppt)		Mean mortality in 45 ppt treatment during exposure and revival period	
	N	%	N	%	N	%
6	60	100	0	0	60	100

Table 26: Analysis of variances with salinity, time, interactions and survival rate of juvenile *Perna viridis*

Source		Type I SS	df	Mean Square	F	Sig.
Salinity	Hypothesis	94.98	2	47.49	46.64	0.00**
	Error	12.22	12	1.018a		
Time	Hypothesis	6.11	6	1.02	1	0.5 NS
	Error	6.11	6	1.018b		
Salinity X Time	Hypothesis	6.11	6	1.02	37.58	0.00**
	Error	0.76	28	0.027c		

**** Significant NS: Not significant**

The effect of salinity-time interaction and within salinity in juvenile *P. viridis* was highly ($P < 0.01$) significant

4.4.2 Response to temperature variation

The juvenile *P. viridis* showed 100 % survival in temperature up to 34°C for 96 hours during sudden exposure. At 36°C and 37°C, 100 % mortality was observed in 12 hours and in 38°C within 6 hours 100 % mortality was recorded (Tables 27, 28 and 29). This indicates that juvenile *P. viridis* are highly vulnerable to higher temperature.

Climate related resilience: The study indicated that seed mussels are vulnerable to temperature rise and will succumb to increased temperature in a day.

Table 27: Mean mortality of juvenile green mussel (*Perna viridis*) on sudden exposure to 36°C temperature and subsequent revival in ambient temperature (32°C)

Time (hrs)	Mortality during exposure in 36°C (N = number,60)		Mortality during revival in ambient temperature (32°C)		Mortality in 36°C treatment during exposure and revival period	
	N	%	N	%	N	%
6	31.3 ± 3.4	52.2 ± 5	0	0	31.3 ± 3.4	52.2 ± 5
12	60	100	0	0	60	100

Table 28: Mean mortality of juvenile green mussel (*Perna viridis*) on sudden exposure to 37°C temperature and subsequent revival in ambient temperature (32°C)

Time (hrs)	Mortality during exposure in 37°C (N = number,60)		Mortality during revival in ambient temperature (32°C)		Mortality in 37°C treatment during exposure and revival period	
	N	%	N	%	N	%
6	44.7 ± 2.9	74.4 ± 4.8	2 ± 2.2	3.3 ± 3.6	46.7 ± 0.9	77.8 ± 1.6
12	60	100	0	0	60	100

Table 29: Mean mortality of juvenile green mussel (*Perna viridis*) on sudden exposure to 38°C temperature and subsequent revival in ambient temperature (32°C)

Time (hrs)	Mortality during exposure in 38°C (N = number,60)		Mortality during revival in ambient temperature (32°C)		Mortality in 38°C treatment during exposure and revival period	
	N	%	N	%	N	%
6	60	100	0	0	60	100

Table 30. Analysis of variance in the juvenile *Perna viridis* under different conditions of survival rate, temperature and time

Source		Type I SS	df	Mean Square	F	Sig.
Temperature	Hypothesis	6.93	3	2.31	1	0.53 NS
	Error	4.69	2.03	2.309a		
Time	Hypothesis	4.56	1	4.56	3.86	0.19 NS
	Error	2.36	2	1.182b		
Temperature X Time	Hypothesis	2.36	2	1.18	1233.2	0.00 **
	Error	0.01	12	0.001c		

**** Significant NS: Not significant**

No significance was observed in *P. viridis* with temperature and time whereas temperature – time interaction was highly significant ($P < 0.01$)

4.5 Adult *Perna viridis* (Green mussel)

Adults of *P. viridis* of average length $35.79 \text{ mm} \pm 2.633 \text{ SD}$, width $33.79 \text{ mm} \pm 1.35 \text{ SD}$ and thickness $31.79 \text{ mm} \pm 1.33 \text{ SD}$ with average total weight $28.8 \text{ gm} \pm 1.63 \text{ SD}$ were used for the experiments. The dissolved oxygen level was 7.2 mg L^{-1} . The control salinity was 10 ppt, ambient temperature was 30°C and pH was 7.52 throughout the experimental period.

4.5.1 Response to salinity variation

Adult *P. viridis* could tolerate exposure in salinities 25, 30 and 40 ppt upto 96 hours when transferred suddenly 35 ppt. There was 100 % survival during this period. But in salinities like 5, 10, 15, 20 ad 45 ppt they could not survive for more than 12 hours (Tables 31 to 35). At higher and lower salinities they could not form byssal thread.

Climate based resilience: The study indicated that adult green mussels were vulnerable to sudden exposure to low salinities from 20 ppt onwards and higher salinity of 45 ppt .They had resilience for a narrow range of salinity change when they were in an ambient salinity of 35 ppt. They could survive 10 ppt lower than 35 ppt and just 5 ppt higher. The results will be beneficial to mussel farmers. When the salinity in the farm area drops below 25 ppt it is better to harvest immediately. The harvest will have to be done within 12 hrs if the salinity goes below 25 ppt. Similarly if salinity is more than 40 ppt, then also it is better to harvest immediately.

Table 31. Mean mortality of adult green mussel (*Perna viridis*) on sudden exposure to 5 ppt salinity and subsequent revival in ambient salinity (35 ppt).

Time (hrs)	Mortality during exposure in 5ppt (N = number,60)		Mortality during revival in ambient salinity (35 ppt)		Mortality in 5 ppt treatment during exposure and revival period	
	N	%	N	%	N	%
6	9.3 ± 2.5	15.5 ± 4.2	0	0	9.3 ± 2.5	15.5 ± 4.2
12	60	100	0	0	60	100

Table 32. Mean mortality of adult green mussel (*Perna viridis*) on sudden exposure to 10 ppt salinity and subsequent revival in ambient salinity (35 ppt).

Time (hrs)	Mortality during exposure in 10 ppt (N = number,60)		Mortality during revival in ambient salinity (35 ppt)		Mortality in 10 ppt treatment during exposure and revival period	
	N	%	N	%	N	%
6	13.3 ± 2.5	22.2 ± 4.2	0	0	13.3 ± 2.5	22.2 ± 4.2
12	60	100	0	0	60	100

Table 33. Mean mortality of adult green mussel (*Perna viridis*) on sudden exposure to 15 ppt salinity and subsequent revival in ambient salinity (35 ppt)

Time (hrs)	Mortality during exposure in 15 ppt (N = number,60)		Mortality during revival in ambient salinity (35 ppt)		Mortality in 15 ppt treatment during exposure and revival period	
	N	%	N	%	N	%
6	16.3 ± 3.3	27.2 ± 5.5	7 ± 2.4	11.7 ± 4.1	23.3 ± 4.8	38.9 ± 8.0
12	60	100	0	0	60	100

Table 34. Mean mortality of adult green mussel (*Perna viridis*) on sudden exposure to 20 ppt salinity and subsequent revival in ambient salinity (35 ppt)

Time (hrs)	Mortality during exposure in 20 ppt (N = number,60)		Mortality during revival in ambient salinity (35 ppt)		Mortality in 20 ppt treatment during exposure and revival period	
	N	%	N	%	N	%
6	24.7 ± 3.9	41.1 ± 6.4	12.7 ± 2.5	21.1 ± 4.2	37.3 ± 3.4	62.2 ± 3.4
12	60	100	0	0	60	100

Table 35. Mean mortality of adult green mussel (*Perna viridis*) on sudden exposure to 45 ppt salinity and subsequent revival in ambient salinity (35 ppt)

Time (hrs)	Mortality during exposure in 45 ppt (N = number,60)		Mortality during revival in ambient salinity (35 ppt)		Mortality in 45 ppt treatment during exposure and revival period	
	N	%	N	%	N	%
6	31.3 ± 3.4	52.2 ± 5.7	21 ± 3.7	35 ± 6.2	52.3 ± 5.2	87.2 ± 8.6
12	60	100	0	0	60	100

Table 36. Analysis of variance in the adult *Perna viridis* under different conditions of time, salinity and survival rate

Source		Type I SS	df	Mean Square	F	Sig.
Salinity	Hypothesis	20.96	5	4.19	1	0.63 NS
	Error	4.55	1.08	4.192a		
Time	Hypothesis	20.12	1	20.12	96.3	0.00**
	Error	0.84	4	0.209b		
Salinity X Time	Hypothesis	0.84	4	0.21	17.68	0.00**
	Error	0.24	20	0.012c		

**** Significant NS: Not significant**

Salinity-time interaction and within salinity was highly significant ($P < 0.01$)

4.5.2 Response to temperature variation

Survival of adult *P. viridis* was 100 % in 32 to 34°C up to 96 hours. At 36°C within 24 hours 84.3 % ± 7.5 SD mortality was recorded and in 48 hours it was 100 % (Table 37). But in 37°C 100 % mortality was recorded within 36 hours (Table 38). One degree higher temperature from 37°C could not be tolerated by adult *P. viridis*, and in 6 hours there was 100 % mortality (Table 39). This indicates that adult *P. viridis* is vulnerable to higher temperature. In 32 to 34°C within 6 hours they formed byssal thread. The favourable condition for the byssal thread formation of adults and juveniles are same (32 to 34°C).

85

Climate based resilience: The study indicated that adult green mussel were highly vulnerable to sudden increase in temperature from 32°C. They were resilient only for 2°C increase in temperature. This information is useful to mussel farmers. In summer the farmed stock may be affected if the temperature increases to above 34°C. Hence all precautions to bring down the temperature should be taken

Table 37. Mean mortality of adult green mussel (*Perna viridis*) on sudden exposure to 36°C temperature and subsequent revival in ambient temperature (32°C)

Time (hrs)	Mortality during exposure in 36°C (N = number,60)		Mortality during revival in ambient temperature (32°C)		Mortality in 36°C treatment during exposure and revival period	
	N	%	N	%	N	%
36	30.3 ± 4.5	50.5 ± 7.5	0	0	50.6 ± 2.6	84.3 ± 7.5
48	60	100	0	0	60	100

Table 38. Mean mortality of adult green mussel (*Perna viridis*) on sudden exposure to 37°C temperature and subsequent revival in ambient temperature (32°C).

Time (hrs)	Mortality during exposure in 37°C (N = number,60)		Mortality during revival in ambient temperature (32°C)		Mortality in 37°C treatment during exposure and revival period	
	N	%	N	%	N	%
6	45.3 ± 2.1	75.6 ± 3.4	2 ± 2.2	3.3 ± 3.6	47.3 ± 0.9	78.9 ± 1.6
12	51 ± 3.7	85 ± 6.2	5 ± 2.2	8.3 ± 3.6	56 ± 1.6	93.3 ± 2.7
24	59.3 ± 0.9	98.9 ± 1.6	0	0	60	100



Table 39. Mean mortality of adult green mussel (*Perna viridis*) on sudden exposure to 38°C temperature and subsequent revival in ambient temperature (32°C).

Time (hrs)	Mortality during exposure in 38°C (N = number,60)		Mortality during revival in ambient temperature (32°C)		Mortality in 38°C treatment during exposure and revival period	
	N	%	N	%	N	%
6	60	100	0	0	60	100

Table 40. Analysis of variance in the adult *Perna viridis* under different conditions of temperature, time and survival rate

Source		Type I SS	df	Mean Square	F	Sig.
Temperature	Hypothesis	18.32	3	6.11	4.42	0.03*
	Error	12.78	9.25	1.382a		
Time	Hypothesis	9.8	4	2.45	2.89	0.009**
	Error	6.79	8	.849b		
Temperature X Time	Hypothesis	6.79	8	0.85	222.2	0.00**
	Error	0.11	30	0.04c		

**** Significant**

The analysis of variance showed ($P < 0.05$) significance within temperature differences and the temperature-time interaction was observed to be highly significant ($P < 0.01$) to the survival rate.

4.6 Juvenile *Villorita cyprinoides* (Black clam)

Juvenile *V. cyprinoides* of average length 26.7mm \pm 1.68 SD, width 29.7mm \pm 1.90 SD and thickness 38.7 mm \pm 10.44 SD with average total weight 20.5 gm \pm 0.36 SD were used for the experiments.

The dissolved oxygen level was 7.4 mg L⁻¹. The control salinity was 10 ppt, ambient water temperature 31°C, water temperature 32°C and pH 7.52 throughout the experimental period.

4.6.1 Response to salinity variation

The survival rate of juvenile *Villorita cyprinoides* in 5 to 30 ppt when suddenly transferred from 10 ppt was about 100 % up to 96 hours. At 35 ppt salinity up to 36 hours 100 % survival was recorded (Table 41). In 35 ppt up to 36 hrs, there was no mortality. After that at 48 and 72 hrs there was 17.2 % \pm 4.8 SD and 36.7 % \pm 6.2 SD respectively. In 96 hours, there was 100 % mortality. At 40 ppt 78.3 % \pm 10 SD and 86.1 % \pm 9.7 SD mortality was observed in 24 and 36 hrs (Table 42). In 48 hours, there was 100 % mortality. At 45 ppt, with in 12 hour 100 % mortality was recorded (Table 43).

Climate based resilience: Juvenile *V. cyprinoides* had high resilience capacity. From 10 ppt they could survive up to 30 ppt without any mortality. The clams were vulnerable to higher salinities like 35 ppt and above.

Table 41. Mean mortality of juvenile black clam (*Villorita cyprinoides*) on sudden exposure to 35 ppt salinity and subsequent revival in ambient salinity (10 ppt)

Time (hrs)	Mortality during exposure in 35 ppt (N = number,60)		Mortality during revival in ambient salinity (10 ppt)		Mortality in 35 ppt treatment during exposure and revival period	
	N	%	N	%	N	%
48	10.3 \pm 2.9	17.2 \pm 4.8	0	0	10.3 \pm 2.9	17.2 \pm 4.8
72	22 \pm 3.7	36.7 \pm 6.2	0	0	22 \pm 3.7	36.7 \pm 6.2
96	60	100	0	0	60	100

Table 42. Mean mortality of juvenile black clam (*Villorita cyprinoides*) on sudden exposure to 40 ppt salinity and subsequent revival in ambient salinity (10 ppt)

Time (hrs)	Mortality during exposure in 40 ppt (N = number,60)		Mortality during revival in ambient salinity (10 ppt)		Mortality in 40 ppt treatment during exposure and revival period	
	N	%	N	%	N	%
24	16.3 ± 2.9	27.2 ± 4.8	30.7 ± 3.3	51.1 ± 5.5	47 ± 6.2	78.3 ± 10
36	49 ± 4.5	81.7 ± 7.6	2.7 ± 1.2	4.4 ± 8.3	51.7 ± 5.8	86.1 ± 9.7
48	58.3 ± 9.7	97.2 ± 2.1	1.7 ± 1.2	2.7 ± 2.1	60	100

Table 43. Mean mortality of juvenile black clam (*Villorita cyprinoides*) on sudden exposure to 45 ppt salinity and subsequent revival in ambient salinity (10 ppt)

Time (hrs)	Mortality during exposure in 45 ppt (N=number,60)		Mortality during revival in ambient salinity (10 ppt)		Mortality in 45 ppt treatment during exposure and revival period	
	N	%	N	%	N	%
6	24 ± 3.3	40 ± 5.5	3 ± 1.6	5 ± 2.7	33 ± 2.8	55 ± 4.7
12	60	100	0	0	0	0

Table 44. Analysis of variance in the juvenile *Villorita cyprinoides* under different conditions of survival rate, salinity, and time

Source		Type I SS	df	Mean Square	F	Sig.
Salinity	Hypothesis	77.22	3	25.74	13.48	0.00**
	Error	22.32	11.69	1.909a		
Time	Hypothesis	21.9	6	3.65	3.81	0.03*
	Error	10.55	11	.959b		
Salinity X Time	Hypothesis	10.55	11	0.96	61.26	0.00*
	Error	0.63	40	0.016c		

** Significant

The salinity-time interaction and within salinity was highly significant (P < 0.01)

4.6.2 Response to temperature variation

The juvenile *V. cyprinoides* which were in an ambient temperature of 32°C when exposed to higher temperature without acclimatization survived up to 96 hours in temperature 34 to 40°C. At 42°C up to 36 hours 100 % survival was recorded (Table 45). Mortality of 57.8 % ± 8.8 SD was recorded in 48 hours and increased to 100 % within 72 hours.

Climate based resilience: Juvenile *V. cyprinoides* were resilient to sudden variation in temperature of up to 8°C when the ambient temperature was 32°C .Beyond this they were vulnerable.

Table 45. Mean mortality of juvenile black clam (*Villorita cyprinoides*) on sudden exposure to 42°C temperature and subsequent revival in ambient temperature (32°C)

Time (hrs)	Mortality during exposure in 42°C (N = number,60)		Mortality during revival in ambient temperature (32°C)		Mortality in 42°C treatment during exposure and revival period	
	N	%	N	%	N	%
48	31.7 ± 4.6	52.8 ± 7.7	3 ± 1.6	5 ± 2.7	34.7 ± 5.3	57.8 ± 8.8
72	60	100	0	0	60	100

4.7. Adult *Villorita cyprinoides* (Black clam)

Adult *V. cyprinoides* of average length 43.6 mm ± 2.7 SD, width 40.3 mm ± 2.1 SD and thickness 48.2 mm ± 1.6 SD with average total weight 30gm ± 1.6 SD were used for the experiments.

The dissolved oxygen level was 7.4 mg L⁻¹ and ammonia was 0.58 µmol/L. The control salinity was 10 ppt, ambient water temperature 31°C was water temperature was 32°C and pH was 7.52 throughout the experimental period.

4.7.1 Response to salinity variation

The adult *V. cyprinoides* showed 100 % survival in salinities up to 30 ppt for 96 hours on sudden exposure from 10 ppt. However in 35 ppt the clam suffered 52.2 % \pm 4.8 SD 81.7 % \pm 6.2 SD ad 100 % mortality in 6, 12 and 24 hours (Table 46). The mortality was higher and faster in 40 and 45 ppt salinities when all the clams suffered mortality in 12 hours (Tables 47 and 48).

Climate based resilience: The study indicated that adult *V. cyprinoides* were very resilient and could tolerate up to 20 ppt sudden increase in salinity without bay mortality for 96 hours. Subsequently in 35 ppt they became vulnerable.

Table 46. Mean mortality of adult black clam (*Villorita cyprinoides*) on sudden exposure to 35 ppt salinity and subsequent revival in ambient salinity (10 ppt)

Time (hrs)	Mortality during exposure in 35 ppt (N=number,60)		Mortality during revival in ambient salinity (10 ppt)		Mortality in 35 ppt treatment during exposure and revival period	
	N	%	N	%	N	%
6	31.3 \pm 2.9	52.2 \pm 4.8	0	0	31.3 \pm 2.9	52.2 \pm 4.8
12	49 \pm 3.7	81.7 \pm 6.2	0	0	49 \pm 3.7	81.7 \pm 6.2
24	60	100	0	0	60	100

Table 47. Mean mortality of adult black clam (*Villorita cyprinoides*) on sudden exposure to 40 ppt salinity and subsequent revival in ambient salinity (10 ppt)

Time (hrs)	Mortality during exposure in 40 ppt (N=number,60)		Mortality during revival in ambient salinity (10 ppt)		Mortality in 40 ppt treatment during exposure and revival period	
	N	%	N	%	N	%
6	45.3 \pm 3.6	75.6 \pm 2.6	10.3 \pm 3.2	17.2 \pm 5	55.7 \pm 0.4	92.8 \pm 0.7
12	57.7 \pm 2.6	96.1 \pm 4.3	2.3 \pm 2.6	3.9 \pm 4.3	60	100

Table 48. Mean mortality of adult black clam (*Villorita cyprinoides*) on sudden exposure to 45 ppt salinity and subsequent revival in ambient salinity (10 ppt)

Time (hrs)	Mortality during exposure in 45 ppt (N=number,60)		Mortality during revival in ambient salinity (10 ppt)		Mortality in 45 ppt treatment during exposure and revival period	
	N	%	N	%	N	%
6	50.3 ± 2.8	83.9 ± 4.7	9.7 ± 2.8	16.1 ± 4.7	60	100
12	60	100	0	0	60	100

Table 49. Analysis of variance in the adult *Villorita cyprinoides* under different conditions of salinity, time and survival rate

Source		Type I SS	df	Mean Square	F	Sig.
Salinity	Hypothesis	9.11	3	3.04	3.07	0.13 NS
	Error	5.02	5.08	0.989a		
Time	Hypothesis	3.17	2	1.59	2.3	0.22 NS
	Error	2.76	4	0.690b		
Salinity X Time	Hypothesis	2.76	4	0.69	131.58	0.00**
	Error	0.09	18	0.05c		

**** Significant NS: Not Significant**

The salinity – time interaction was very significant ($P < 0.01$) in the adult black clam, *Villorita cyprinoides*.

4.7.2 Response to temperature variation

Adult black clam, *V. cyprinoides* showed 100% survival when they were exposed to temperatures from 32 to 38°C upto 96 hours. At 40°C and 42°C the *V. cyprinoides* show survival of 100 % up to 72 hours (Table 50 and Table 51). At 48 hours, in 40°C, 82.2 % ± 7.7 SD mortality and in 42°C 96.7 % ± 4.7 mortality was recorded. The mortality was 100 % in both of treatments at 72 hours. While, comparing juvenile and adults, no mortality was recorded in juveniles at 40°C.

Climate based resilience: Adult *V. cyprinoides* were resilient to higher temperature exposures from 32°C for about 6°C (up to 38°C). The clam was vulnerable to 40°C and 42°C exposures.

Table 50. Mean Mortality of adult black clam (*Villorita cyprinoides*) on sudden exposure to 40°C temperature and subsequent revival in ambient temperature (32°C)

Time (hrs)	Mortality during exposure in 40°C (N=number,60)		Mortality during revival in ambient temperature (32°C)		Mortality in 40°C treatment during exposure and revival period	
	N	%	N	%	N	%
6	49.3 ± 4.6	82.2 ± 7.7	0	0	49.3 ± 4.6	82.2 ± 7.7
12	60	100	0	0	60	100

Table 51. Mean mortality of adult black clam (*Villorita cyprinoides*) on sudden exposure to 42°C temperature and subsequent revival in ambient temperature (32°C)

Time (hrs)	Mortality during exposure in 42°C (N=number,60)		Mortality during revival in ambient temperature (32°C)		Mortality in 42°C treatment during exposure and revival period	
	N	%	N	%	N	%
48	48 ± 1.6	80 ± 2.6	10 ± 1.6	16.7 ± 2.7	58 ± 2.8	96.7 ± 4.7
72	60	100	0	0	60	100

Table 52. Analysis of variance in the adult *Villorita cyprinoides* under different conditions of temperature, time, and survival rate

Source		Type I SS	df	Mean Square	F	Sig.
Temperature	Hypothesis	77.128	2	38.564	16.346	0.005**
	Error	12.794	5.423	2.359a		
Time	Hypothesis	22.634	5	4.527	23.598	0.002**
	Error	0.959	5	0.192b		
Temperature X Time	Hypothesis	0.959	5	0.192	5.976	0.001**
	Error	0.77	24	0.032c		

**** Significant**

In general, the survival rate of adult *Villorita cyprinoides* in response to 40°C and 42°C was highly significant ($P < 0.01$).

4.8 Comparison of resilience of different species to salinity stress

The series of extreme event simulation experiments showed that the adult and young ones of different species have varied resilience capacity. Juvenile of *E. suratensis*, post larvae of *P. monodon*, and juvenile and adult *V. cyprinoides* were resilient to sudden lowering of salinity. However, they could not tolerate salinities above 35ppt.

Juvenile of *E. suratensis* and PL of *P. monodon* were resilient up to 35 ppt but became vulnerable to salinity based stress higher than 35 ppt.

Juvenile of *T. blochii* and adult and juvenile mussel *P. viridis* were vulnerable to sudden lowering of salinity below 20 ppt. Similarly they could not tolerate higher salinities of 40 ppt. Adult mussel were more resilient to higher temperature than seed mussel.

4.8.1 Comparison of resilience of different species to salinity stress by correlation

Villorita cyprinoides (Black clam, Adult and Juvenile), *Eetroplus suratensis* (Pearl spot) and *Penaeus monodon* (Tiger shrimp) was resilient to the hyper-saline

conditions (35 to 45 ppt). *Perna viridis* (Adult green mussel) and *Trachinotus blochii* (silver pompano) were observed to be showing higher mortality rate in both the low-saline (5 to 20 ppt) and hyper-saline (40 to 45 ppt) conditions. This observation proves that these two species are active with the ambient salinities but tend to be vulnerable to the exposure of extreme stress levels. Correlation analysis (Table 53) were performed to substantiate the results of significance relationship between the varying salinities and survival rate of each species. *Etroplus suratensis* and *Penaeus monodon*, *Perna viridis* (juvenile) and *Trachinotus blochii*, *Villorita cyprinoides* (juvenile) and *Etroplus suratensis*, *Villorita cyprinoides* (juvenile) and *Penaeus monodon*, was noted to have positive correlation of 0.9 to 1 significance level. The percentage average survival rate of each species and correlation analysis of survival rate within each species to the exposure of different salinities was portrayed in (Fig.15 and Table 53) respectively. From the observations, only three species: *Villorita cyprinoides*, *Etroplus suratensis* and *Penaeus monodon* are found to be adaptive and resilient to the low-medium salinities whereas vulnerable to the extreme saline conditions. Other species such as *Perna viridis* and *Trachinotus blochii* are confined to the ambient saline conditions (20-35 ppt) for active growth.

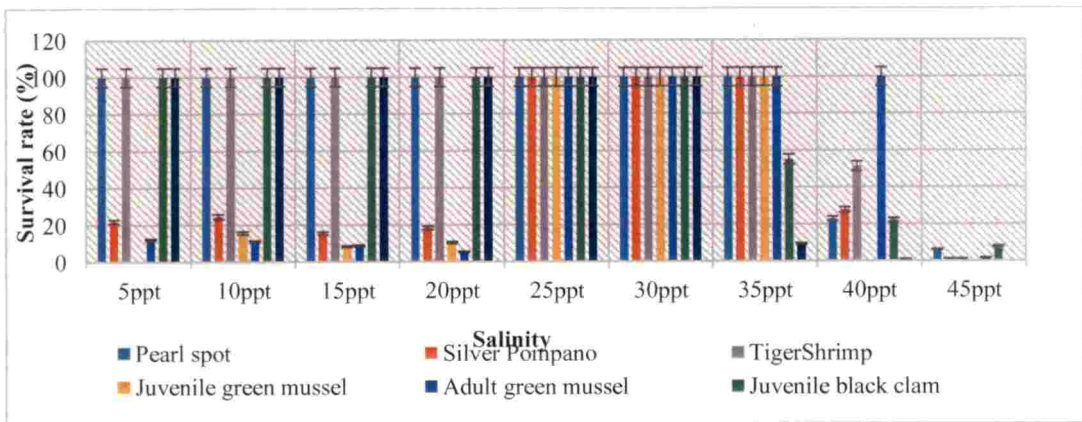


Fig 3: Average percentage survival rate of different species with exposure to varying salinities (5 to 45 ppt) irrespective of time

Table. 53. Correlation analysis between different species and their exposure to varying salinities (5 to 45 ppt)

Species	Pearl spot	Silver pompano	Tiger shrimp	Juvenile green mussel	Adult green mussel	Juvenile black clam	Adult black clam
Pearl spot	1.0						
Silver pompano	0.4	1.0					
Tiger shrimp	1.0	0.4	1.0				
Juvenile green mussel	0.4	1.0	0.4	1.0			
Adult green mussel	0.0	0.8	0.1	0.7	1.0		
Juvenile black clam	0.9	0.1	0.9	0.2	-0.3	0.0	
Adult black clam	0.8	-0.1	0.7	0.0	-0.4	1.0	1.0

4.9 Comparison of resilience of different species to temperature stress

Resilience to heat stress was the highest for juvenile pearl spot and juvenile black clam. They could survive even in 40°C. Above this temperature they became vulnerable. This was followed by adult black clam which was resilient to 38°C. Juvenile of silver pompano and seed and adult of green mussel were the least tolerant to higher temperature stress.

4.9.1 Comparison of resilience of different species to temperature stress by Correlation

Villorita cyprinoides (Black clam, adult and juvenile) and *Etroplus suratensis* (Juvenile pearl spot) showed high resilience to the exposure of extreme temperatures (40°C-42°C). *Perna viridis* (Adult green mussel) is moderately vulnerable to the high temperatures. The species such as *Trachinotus blochii* (silver pompano), *Penaeus monodon* (Tiger shrimp) and *Perna viridis* (Green mussel, juvenile) are vulnerable to exceeding temperature (40°C-42°C). The significance relationship within species was statistically analysed using correlation represented

in (Table 54). *E. suratensis* and juvenile *V. cyprinoides* showed positive correlation at +1 significance level, similarly, *T. blochii* and *P. monodon* also showed positive correlation to different temperatures (30°C-42°C). From the graphical representation (Fig.16) and correlation analysis, *E.suratensis* and *V. cyprinoides* are the two species observed to be extremely adaptive and resilient to high temperature with good survival rate.

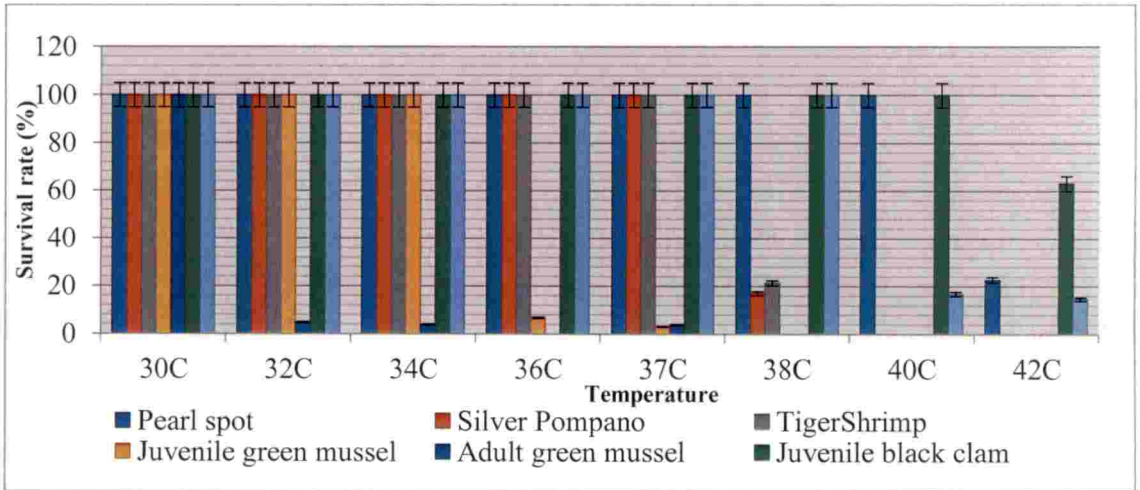


Fig 4: Average percentage survival rate of different species with exposure to varying temperature (30°C to 42°C) irrespective of time

Table 54. Correlation analysis between different species and their exposure to varying temperatures (30°C to 42°C)

Species	Pearl spot	Silver pompano	Tiger shrimp	Juvenile green mussel	Adult green mussel	Juvenile black clam	Adult black clam
Pearl spot	1.00						
Silver pompano	0.53	1.00					
Tiger shrimp	0.54	1.00	1.00				
Juvenile green mussel	0.31	0.63	0.63	1.00			
Adult green mussel	0.16	0.33	0.33	0.53	1.00		
Juvenile black clam	1.00	0.53	0.54	0.31	0.16	1.00	
Adult black clam	0.67	0.81	0.83	0.47	0.25	0.67	1.00

Table 55 : Resilience capacity of test animals in relation to temperature stress duration from day 1 to 4 days per category of mortality percentage

4 days (96hrs)	30°C	32°C	34°C	36°C	37°C	38°C	40 °C	42°C
<i>E. suratensis</i>	C							
<i>T. blochii</i>	C							
<i>P. monodon</i>	C							
<i>P. viridis</i> (J)		C						
<i>P. viridis</i> (A)		C						
<i>V. cyprinoides</i> (J)		C						
<i>V. cyprinoides</i> (A)		C						
3 days (72hrs)	30°C	32°C	34°C	36°C	37°C	38°C	40 °C	42°C
<i>E. suratensis</i>	C							
<i>T. blochii</i>	C							
<i>P. monodon</i>	C							
<i>P. viridis</i> (J)		C						
<i>P. viridis</i> (A)		C						
<i>V. cyprinoides</i> (J)		C						
<i>V. cyprinoides</i> (A)		C						
2 days (48hrs)	30°C	32°C	34°C	36°C	37°C	38°C	40 °C	42°C
<i>E. suratensis</i>	C							
<i>T. blochii</i>	C							
<i>P. monodon</i>	C							
<i>P. viridis</i> (J)		C						
<i>P. viridis</i> (A)		C						
<i>V. cyprinoides</i> (J)		C						
<i>V. cyprinoides</i> (A)		C						
1 day (24hrs)	30°C	32°C	34°C	36°C	37°C	38°C	40 °C	42°C
<i>E. suratensis</i>	C							
<i>T. blochii</i>	C							
<i>P. monodon</i>	C							
<i>P. viridis</i> (J)		C						
<i>P. viridis</i> (A)		C						
<i>V. cyprinoides</i> (J)		C						
<i>V. cyprinoides</i> (A)		C						

C: Control

Table 56: Resilience capacity of test animals in relation to salinity stress duration from day 1 to 4 days per category of mortality percentage

4 days (96hrs)	5ppt	10ppt	15ppt	20ppt	25ppt	30ppt	35ppt	40ppt	45ppt
<i>E. suratensis</i>		C							
<i>T. blochii</i>						C			
<i>P. monodon</i>									
<i>P. viridis</i> (J)							C		
<i>P. viridis</i> (A)							C		
<i>V. cyprinoides</i> (J)		C							
<i>V. cyprinoides</i> (A)		C							
3 days (72hrs)	5ppt	10ppt	15ppt	20ppt	25ppt	30ppt	35ppt	40ppt	45ppt
<i>E. suratensis</i>		C							
<i>T. blochii</i>						C			
<i>P. monodon</i>		C							
<i>P. viridis</i> (J)							C		
<i>P. viridis</i> (A)							C		
<i>V. cyprinoides</i> (J)		C							
<i>V. cyprinoides</i> (A)		C							
2 days (48hrs)	5ppt	10ppt	15ppt	20ppt	25ppt	30ppt	35ppt	40ppt	45ppt
<i>E. suratensis</i>		C							
<i>T. blochii</i>						C			
<i>P. monodon</i>									
<i>P. viridis</i> (J)							C		
<i>P. viridis</i> (A)							C		
<i>V. cyprinoides</i> (J)		C							
<i>V. cyprinoides</i> (A)		C							
1 day (24hrs)	5ppt	10ppt	15ppt	20ppt	25ppt	30ppt	35ppt	40ppt	45ppt
<i>E. suratensis</i>		C							
<i>T. blochii</i>						C			
<i>P. monodon</i>									
<i>P. viridis</i> (J)							C		
<i>P. viridis</i> (A)							C		
<i>V. cyprinoides</i> (J)		C							
<i>V. cyprinoides</i> (A)		C							

C: Control

4.10 Response of *Isochrysis galbana* to sudden increase in temperature

The cell density of *I. chrysis* during different periods in 32, 34 and 36°C and during the revival period varied (Fig.17). The cell densities during all the exposure periods from 6 to 96 hrs in 32°C was lower than the cell densities in control temperature of 24°C. During the revival period, the cell densities were comparable with the control salinity density from 6 to 36 hrs but there was considerable difference between the revival cell density and control cell density during 48, 72 and 96 hrs. This indicates that sudden change to 32°C followed by return of ambient temperature retains the capacity of *Isochrysis* to multiply and that it is resilient to 32°C.

The cell density of *I. galbana* in 34°C during the first six hours was lower than the control (Fig.18). But, after that in 12 to 96 hours, the cell densities were much lower than the revival and the control cell densities. However, in the revival treatments after exposure to 34°C, the cell densities were higher than the stress period and were comparable with the control indicating that the stress did not affect the cells completely. They were capable of multiplication and were resilient.

In 36°C the response of *I. galbana* was different from that of 32 and 34°C. The cell densities were much lower during the stress exposure period in all the treatments from 6 to 96 hours (Figure: 19). In the revival phase also, this did not improve and this proved that *I.galbana* is vulnerable to a temperature of 36°C .

4.11 Response to pH variation: sudden exposure

4.11.1 Response of juvenile *Amphiprion perculata* (clown fish) to varied pH stress

Larvae of clown fish *A. percula* of length 0.76 cm \pm 0.19 SD and width 0.27 cm \pm 0.11 SD were found to have behavioural stress when exposed to different pH. In lower pH less than 6.2 they did not survive. There was immediate mortality. In pH 6.40, 6.60 and 6.70 their behaviour changed. They were found to be disoriented and gasping. Finally they were found to rest on the bottom of the experimental container before mortality. Their caudal fin was also affected. There were

aberrations (Plate 12). In pH 6.80, 7.0, 7.2, 7.4, 7.8 and 8.0 the young one of clown fishes were active, they were not affected.



Control pH:8



0- 20 Sec pH:6.44

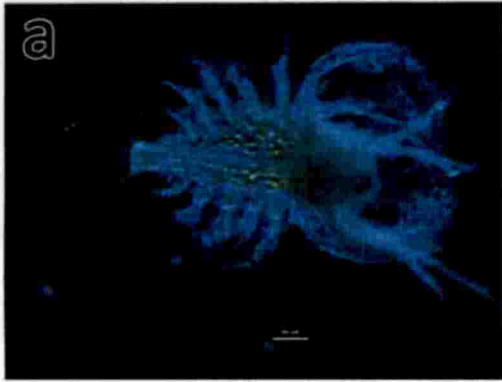


0 -25 Sec pH:6.27

Plate 12: Changes in caudal fin of the *A. percula* on exposure to various pH concentrations

4.11.2 Response of Zooplankton

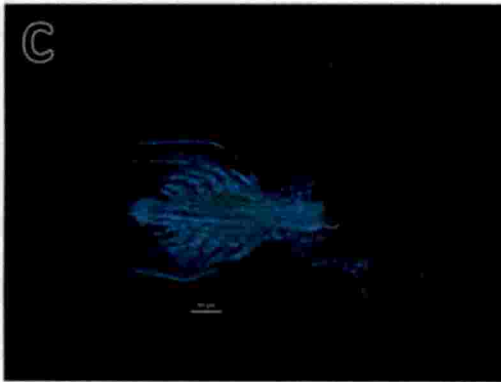
The Cladocerans of proximal length $0.78 \text{ cm} \pm 0.009 \text{ SD}$ and proximal width $0.0041 \text{ cm} \pm 0.0049 \text{ SD}$ were found to be affected to exposure to acidic pH 6.4, 6.6 and 6.7 even for few seconds. The appendages were damaged (Plate 13) and they were found to be disoriented, without swimming capabilities. However in pH 6.8 and above they were found to be active.



Control pH:8



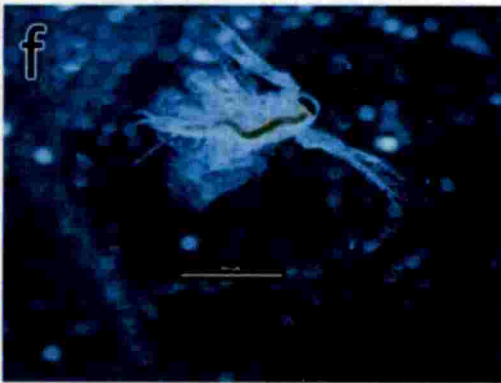
pH:6.78



pH:6.61



pH:6.44



pH:6.27



pH:6.18

Plate 13: Changes in exoskeleton and appendages of the cladoceran on exposure to various pH concentrations

DISCUSSION

CHAPTER 5

DISCUSSION

Marine resources are bearing the brunt of climate change and man-made ecological changes including pollution and overfishing. These affect the ecosystem services and is reflected on the livelihood of fishers and aqua farmers. One of the major impacts of climate change is tremendous increase in frequency of extreme events. In India, also it is expected that there will be increase in droughts and high precipitation. The study conducted to evaluate the response of selected marine resources by simulating extreme abiotic stresses gave some interesting results. It is well known that tolerance of a biota is determined to a large extent by its genetic characteristics. Ecological tolerance is the ability to adapt to environmental variations.

Adaptation is a key word in climate based studies and IPCC defines “Adaptation as the process of adjustment to actual or expected climate and its effects” (IPCC, 2014). Actual adaptation to natural or climate based changes takes decades to happen, but in several geographic ranges marine biota are forced to face sudden environmental stress factors in which some may survive and some may not. One of the sectors which has witnessed huge crop loss due to unpredictable natural events is marine and coastal aquaculture. Most often, species selected for aquaculture are with fast growth rate and high value in domestic market. The adaptive capacity of these resources is not well understood and this creates a situation of helplessness when extreme events occur. In a review on adaptation strategies to climate change, Miller *et al.* (2017) have presented instances of adaptations and maladaptation’s of human systems and marine ecosystems. Only very few studies have been conducted to analyses the response of marine biota to abiotic stress. In the present study the response of different species was to salinity and temperature stress was found to vary.

5.1 Finfish: *Etroplus suratensis*

Etroplus suratensis is basically a brackish water fish. This fish is farmed in ponds as well as in cages in the estuarine region of Kerala and there are farmers who produce seed by natural seed production methods. Hence, there are more people opting to farm pearl spot. In the present study, it was found that pearl spot can tolerate lower ranges of salinity and temperature than the higher values, indicating that it is more prone to drought like situations.

In the experiment conducted by Joy *et al.* (2017). Critical temperature maximum and Critical temperature minimum were observed to be 40 and 14°C respectively.

Extreme events are known to affect fish farmers and in one instance there was a farmer who moved his cages to a location where the environmental conditions would be tolerable to the farmed fish (Chang *et al.*, 2001). This was done during an extreme event where cold water was expected to flow to a farm site. However, he was asked to pay fine for relocating his farm from to an area where he did not have permission. This affected his profit and he had to pay which was actually higher than the losses he would have incurred if he had kept the farm in the same site. This situation is an example of “maladaptation” wherein the actions and the decisions increased the vulnerability of the fish farmer (Miller *et al.*, 2017). It also points to the fact that rules and regulations have to be amended to incorporate responses during extreme climatic events.

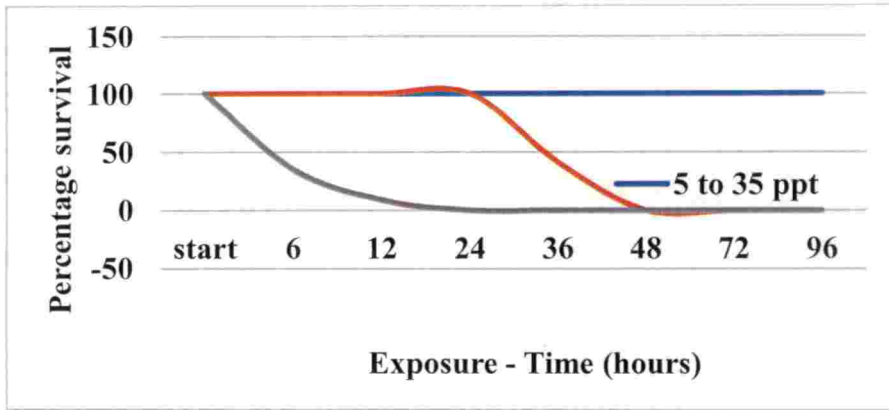


Fig 5: Percentage survival of juvenile *Etroplus suratensis* on sudden exposure to varied salinities for short spells and exposure in ambient salinity for revival

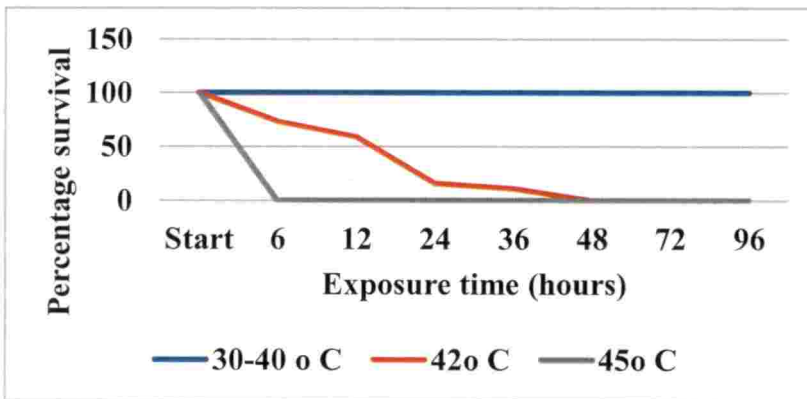


Fig 6: Percentage survival of juvenile *Etroplus suratensis* on sudden exposure to varied temperature for short spells and exposure in ambient temperature for revival

The present study clearly indicated that pearl spot can with stand unexpected stress related to temperature and salinity and this agreed well with the fact that this species is widely distributed in the coastal zones of Kerala, where monsoon related salinity variations are frequent.

5.2 Finfish: *Trachinotus blochii*

Another species of aquaculture importance is the silver pompano. This species is farmed in cages along the coast line of almost all maritime states. This species is also a euryhaline species with tolerance capable of tolerating salinities

between 7 to 58 ppt (Sampaio *et al.*, 2003). Kalidas *et al.*, (2013) observed that the minimum salinity required for survival of juvenile pompano is 4 ppt and below this there was 100% mortality. Kumpf (1971) observed that juvenile of the Florida pompano, *Trachinotus carolinus* were able to survive even in 2 ppt and 45 ppt. For the same species, Main *et al.*, (2008) found that in 5 ppt they had high mortality. They suggested that 10 ppt may be salinity will be better for farming of *T. carolinus*. Studies by Jian-sheng *et al.*, (2008) on *T. ovatus* juveniles indicated that 25 ppt salinity gave good growth. Most of these studies were after acclimatization. However, in the experiments for understanding the response during extreme events, the exposure to salinity treatments is without acclimatization. In a similar study conducted by Moe *et al.*, (1968), they observed that the fishes behaved erratically when transferred to lower salinities directly from higher salinity. They were able to survive only for 7.5 hours. After acclimatization, juvenile pompano were able to survive in lower salinities also (Gunter and Hall, 1963, Perret *et al.* 1971 and Kalidas *et al.*, 2012).

The present study indicates that in 20 and 40 ppt, they are moderately vulnerable. But are vulnerable in lower salinities and higher salinities. Hence farmers have to be cautious and must monitor salinity variations during extreme events and take precautions to prevent mass mortalities. The experiment shows that juvenile *T. blochii* cannot tolerate higher salinities beyond 6 hours.

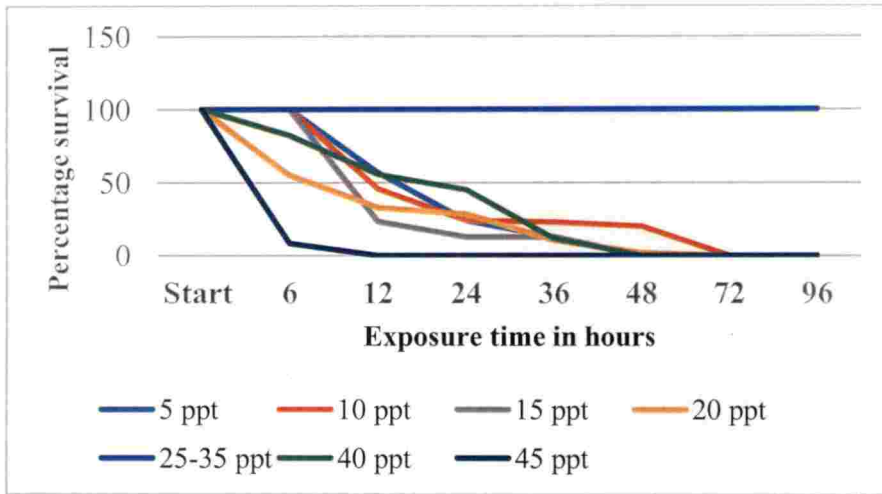


Figure 7: Percentage survival of juvenile *Trachinotus blochii* on sudden exposure to varied salinities for short spells and exposure in ambient salinity for revival

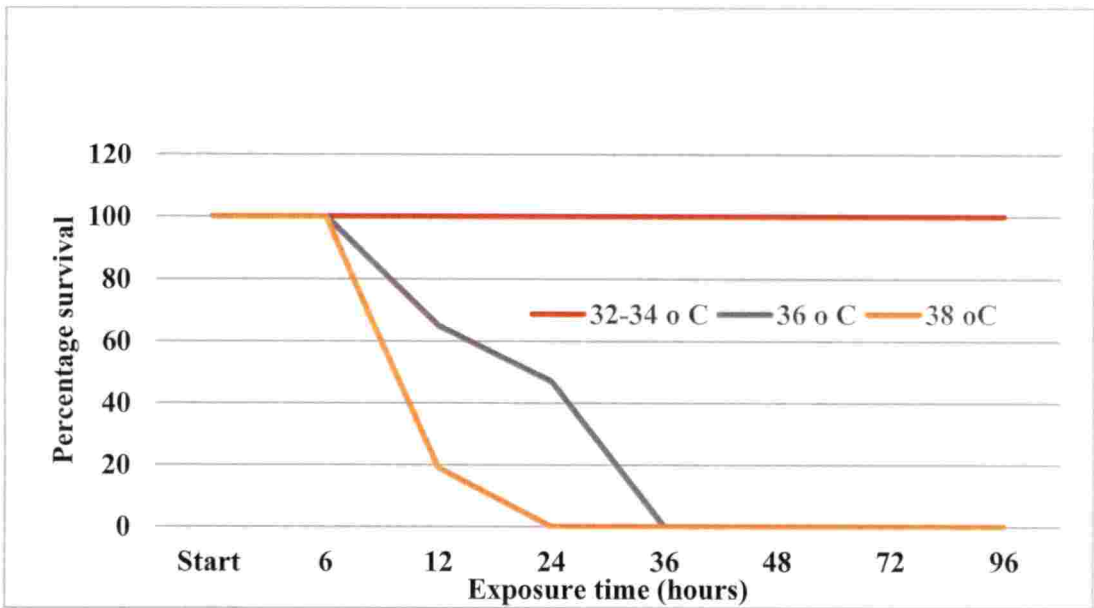


Fig 8: Percentage survival of juvenile *Trachinotus blochii* on sudden exposure to varied temperature for short spells and exposure in ambient temperature for revival

5.3 Shrimp: *Penaeus monodon*

Shrimp farming is an important aquaculture activity in all states of the country. In some maritime states like Kerala, most farming practices are either extensive, that is, without any high investments. These are tide fed ponds and the seed available in the natural water bodies are used for stocking. Sometimes these farmers also convert it to semi-intensive ponds, wherein they stock hatchery produced shrimp larvae. Tiger prawn *P.monodon*, is one of the favourite species of shrimp farmers due to its high market price. They stock PL-21 seed in the ponds. In the present study it was seen that PL-21 of tiger shrimps were not tolerant to high temperatures.

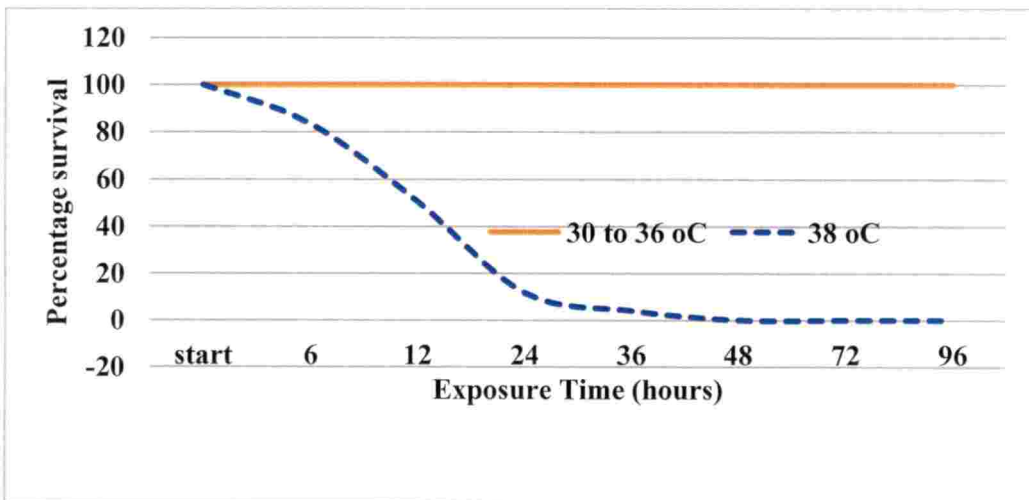


Fig 9: Percentage survival of post larvae of *Penaeus monodon* on sudden exposure to varied temperature and exposure in ambient temperature for revival

Experiments conducted by Villarreal *et al* (1994) showed that the metabolic rate of the larvae *Penaeus vannamei* showed considerable variation with salinity and temperature. They observed that there is a critical point (Cp) which was correlated to temperature and that below this the oxygen consumption of the larvae was reduced. So such metabolic changes would have led to the mortality of the larvae of *P.monodon* in the present experiment. The western school prawn *Metapenaeus dalli* was found to have survival in a very narrow range of temperature and salinity (Crisp *et al.*, 2017). Compared to this species, the resilience of PL 21 of

P.monodon was higher. Kinne (1963, 1964) had indicated that the major environmental factors which affect the survival of larval stages of most crustaceans are temperature and salinity.

Several studies have indicated that variations in salinity affect the shrimp larvae than temperature (Kumlu *et al.* 2000, 2001; Zacharia and Kakati 2004; Ch and Shailender 2013). However, studies have also shown that higher temperatures can affect survival of shrimp larvae (Aktas and Cavdar 2012; Jackson *et al.*, 2003). Zink *et al.* (2013) examined the response of the post larvae and juvenile pink shrimp *Farfantepenaeus duorarum* to combined effect of salinity and temperature on their growth and survival. The survival was found to be affected in hyper saline conditions. In the present study the larvae were not tolerant to higher salinities, though the experimental procedures were different. Similarly, Kumlu and Eroldogan (1997) found that the survival of post larvae of *P. indicus* was lower in higher salinity.

P. monodon is known as white gold because of the high export value and this species is farmed in earthen ponds. Ahmed and Diana (2015a, b) had stated that farming of *P.monodon* and *Macrobrachium rosenbergi* are under threat since surveys in coastal villages have indicated that frequent floods and other climate impacts have increased the vulnerability of this farming practice. They have recommended community based management along with integrated coastal zone management as the strategies for overcoming this challenge. The farmers have opined that changes in salinity and temperature have affected the survival of the stock and this in turn have affected their profit (Ahmed and Diana 2015b). In another study conducted along the southwest coast of Bangladesh, Islam *et al.* (2016) have stated that about 60% shrimp farmers perceived that there has been a sudden change in climate during the last decade and the major change is the high temperature. The farmers were also of the opinion that this high temperature had impacted the farming and resulted in low growth and production. Islam *et al* (2017) have also stated that climate change is affecting the shrimp farmers of Bangladesh and they are resorting to adaptive measures like increasing the pond depth, growing more aquatic plants

and other physical changes to combat climate related challenges. The present study on *P. monodon* is indicating that the larvae can survive in low salinities but higher salinities are not favourable.

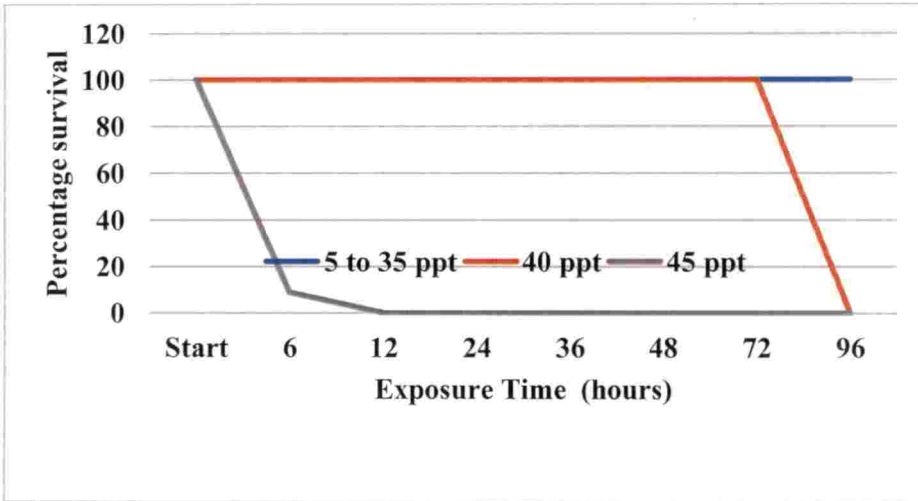


Fig 10: Percentage survival of post larvae of *Penaeus monodon* on sudden exposure to varied salinities for short spells and exposure in ambient salinities for revival

5.4 Green mussel

Critical thermal maximum (CT Max) and minimum (CT Min) are temperatures at which an organism succumbs to death when exposed to high and low temperature from its ambient or acclimatized temperature. Studies related to Critical thermal maximum conducted by Bravo *et al.*, (1998) had shown that *Perna viridis* is more tolerant than *P. perna*, in the same study, low and high lethal salinities were zero and 64 ppt for green mussel which is much lower than that observed in the present study. This may be due to the fact that the present experiments were conducted without acclimatization. The present study indicated that the seed and adult mussels cannot tolerate high salinity as well as low salinities and temperature.

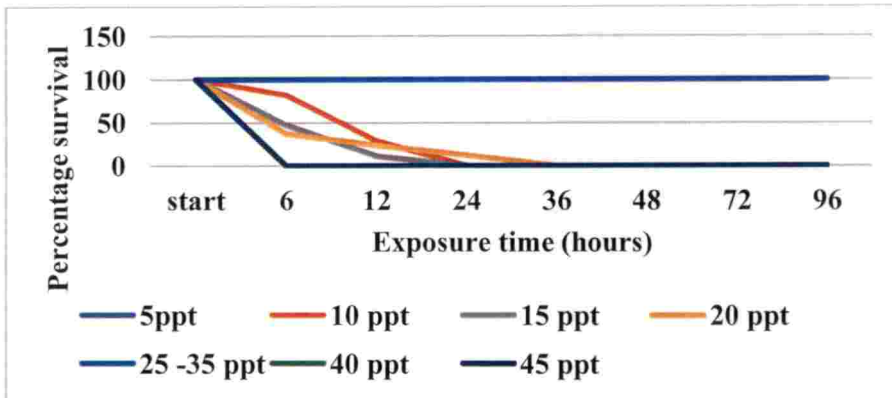


Fig 11: Percentage survival of juvenile *Perna viridis* on sudden exposure to varied salinities for short spells and exposure in ambient salinities for revival

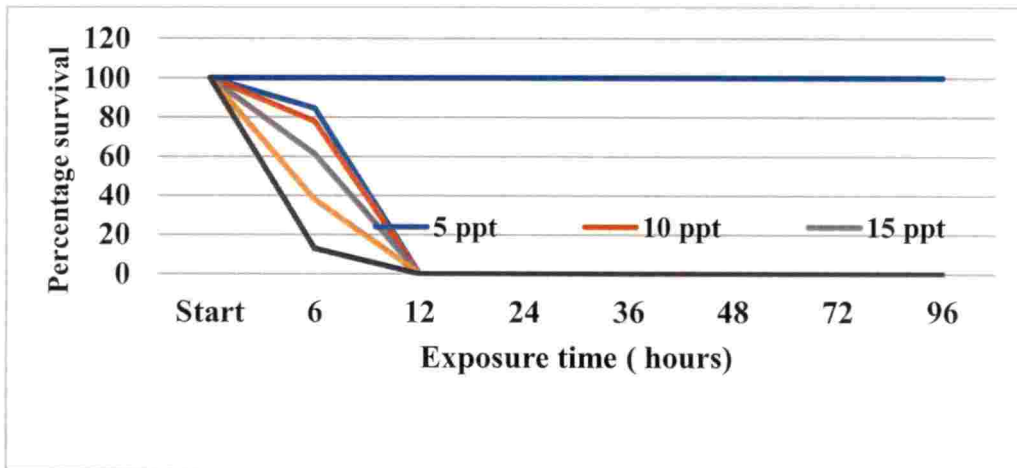


Fig 12: Percentage survival of adult *Perna viridis* sudden exposure to varied salinities for short spells and exposure in ambient salinities for revival

The present study indicated that high rainfall can be detrimental to mussel farmers. It is suggested that mussel seeds are stocked when the salinity stabilizes at 25 ppt. Similarly high salinity stress can also lead to mortality of stock.

In the studies conducted on the filtration rates of the green mussel in different salinities, Rajesh *et al.* (2001) found that the filtration rates varied with salinity and it was highest in 32 ppt, but it was equally high in 25 ppt. This indicates that the physiology of the animal is affected when there is variation in salinity and also that salinity of 25 to 32 ppt were in the preferred range of green mussel. They

also observed that at 15 ppt there was very low filtration and that adult mussels were more tolerant than young mussel. Even the larvae of this species cannot tolerate high temperatures. McFarland *et al.* (2014) observed that *P. viridis* had very low survival below 15 ppt when they were exposed without acclimatization. This is similar to the present study, where even survival at 20 ppt was poor. Manoj and Appukuttan (2003) had observed that the growth of larvae of green mussel increased from a low temperature of 24°C to maximum growth at 31°C. There was total larval mortality at 33°C and 35°C after 24 hours. These reports clearly indicated that the green mussel is vulnerable to temperature stress.

The mussel *Mytella charruana* commonly called 'Sururu' in Brazil was exposed to different salinities (0 to 45) to understand its ability to survive and it was observed that adult mussel (20–54 mm) had high survival in salinities from 2 to 23 ppt, with complete mortality at 0 ppt and 45 ppt (Yuan *et al.*, 2010). Seed of *M. charruana* were able to achieve greater survival with acclimatization. Yuan *et al.*, (2010) have also indicated the threat this species can cause to the ecological functioning of the area where it was introduced. Contrary to this, the mussel *Mytilus galloprovincialis* was found to be highly vulnerable to temperature stress (Anestis *et al.*, 2007).

Just as high thermal stress, mussels in higher latitudes face cold thermal stress from an extreme event. In a study conducted by Firth *et al.* (2011) it was observed that during the winter of 2007 and 2008, *P. viridis* in the Tampa Bay, Florida suffered mortality. This event coincided with extreme weather conditions when atmospheric temperature (AT) became less than 2°C for about 6 hours during low tide. The minimum recorded was 0.53°C and during this period water temperature remained relatively constant about 20°C. The authors are of the opinion that thermal stress caused by exposure to cold at during emersion was the main reason for the mortality event and that such mortality events have occurred in 2009 and 2010. This study clearly indicates the significance of extreme events, even if it is for a short period in causing mortality to inter-tidal animals. Similarly, Wetthey *et al.* (2011) have reported that the cold winter in 2009 to 2010 had a significant impact

on intertidal marine fauna in northern Europe and that warm-adapted native barnacle species had recruitment failure.

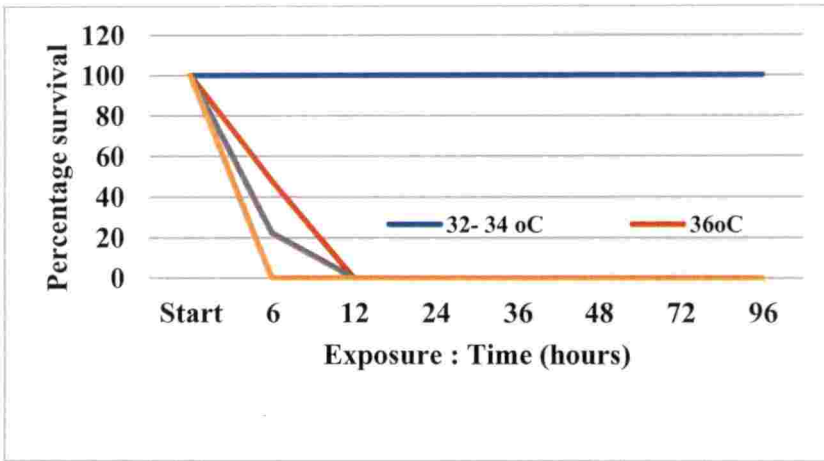


Fig 13: Percentage survival of juvenile *Perna viridis* on sudden exposure to varied temperature for short spells and exposure in ambient temperature for revival

This study indicates that juvenile *P. viridis* are highly vulnerable to higher temperature.

The green mussel *Perna viridis* is a species which is farmed by the rack method in the estuaries of Kerala and Karnataka. In the recent years, there was heavy mortality of the farmed stock in the northern districts and this coincided with the high temperature period of 2015 and 2016. The poor north east monsoon and lack of summer rains led to poor water flow. Such situations can be expected in future also.

The study indicated that adult green mussel were highly vulnerable to sudden increase in temperature from 32°C. They were resilient only for 2°C increase in temperature. This information is useful to mussel farmers. In summer the farmed stock may be affected if the temperature increases to above 34°C

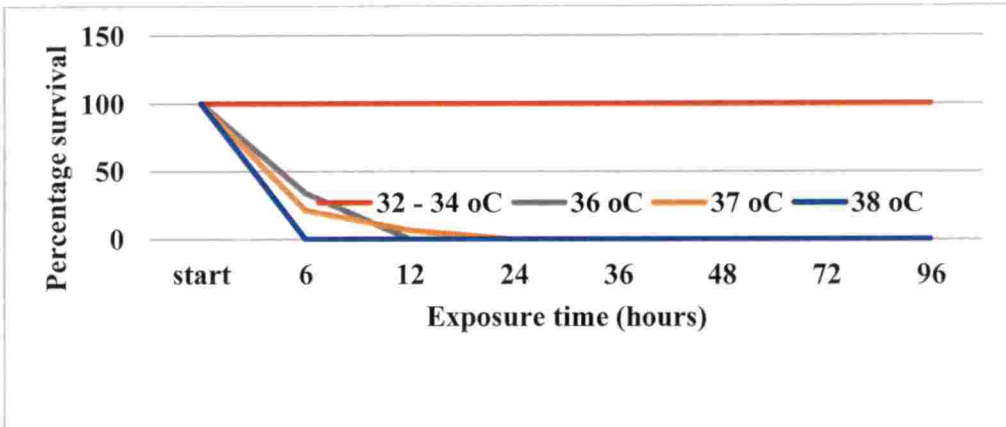


Fig 14: Percentage survival of adult *Perna viridis* on sudden exposure to varied temperatures for short spells and exposure in ambient temperature for revival

5.5 Clam: *Villorita cyprinoides*

The distribution of clams in an estuary is known to be based on salinity variation (Matsuda *et al.* 2008; Xiao *et al.*, 2014). The black clam *V. cyprinoides* is found extensively in the Vembanad Lake in the open water area and also in shrimp ponds. This clam is found mostly in the low saline area. Clams have an important role in an estuary. Their burrowing activity leads to bio-turbation which keeps the benthic ecosystem healthy. Restoration of clam beds which are affected by degradation is also attempted in several areas. The present study indicated that *V. cyprinoides* can survive in extremely low salinities but cannot survive in higher salinities. This indicated that this clam can be affected during droughts. This species is more sensitive to higher salinities and higher temperatures.

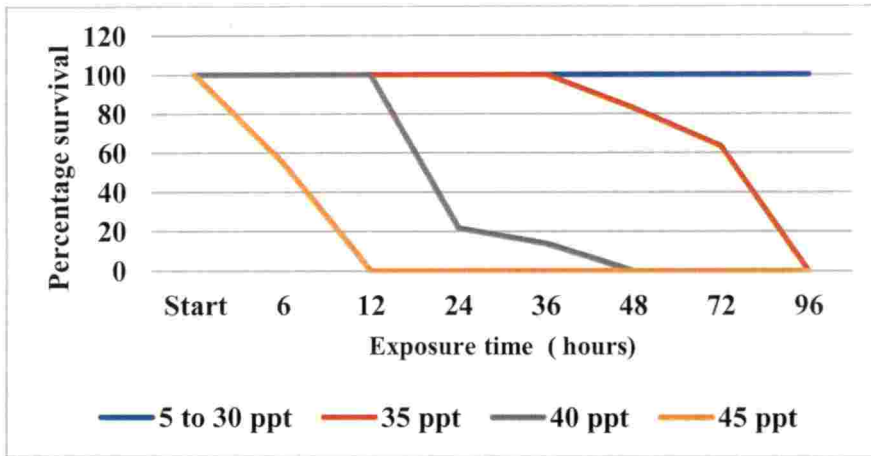


Fig 15: Percentage survival of juvenile *Villorita cyprinoides* sudden exposure to varied temperature for short spells and exposure in ambient temperature for revival

A study conducted by Parada *et al.* (2012) on multispecies mortality in Ulla estuary, Spain during the period 1977-2009 showed that the clams are affected by two types of stress, one related to low salinity during a high tide period and the other the number of days the clams were exposed to a salinities below a threshold level.

In the present study also, it was observed that exposure to higher salinities affected the survival.

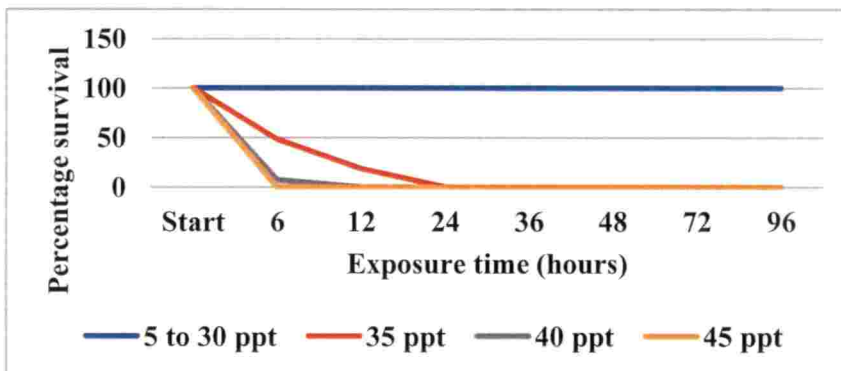


Fig 16: Percentage survival of adult *Villorita cyprinoides* sudden exposure to varied salinities for short spells and exposure in ambient salinities for revival

In an experiment for assessing physiological changes using remote sensing, Macho *et al.* (2016) tried to make models based on remote sense observations,

weather forecasts on extreme events and real time laboratory and field experiments on three species of clams (*Venerupis corrugata*, *Ruditapes decussatus*, *Ruditapes philippinarum*) and one species of cockle (*Cerastoderma edule*). They were able to determine responses and then predict mortality, growth and reproduction of the species which were tested. They also collaborated with the Spanish fisheries partners who wanted to identify the reason for fisheries failure and then develop mitigation measures. Since clam fishing is an activity in which women were actively involved in, they wanted to assess the long term economic effects of climate change.

Xiao *et al.* (2014) observed that in the Asiatic clam *Corbicula fluminea* there was no significant change in different metabolic indices when exposed to different temperature and salinities for a specific size group. However, they found that based on metabolic changes, smaller clams are better for restoration programs since they are more sturdy than bigger clams. In the present study, *V. cyprinoides* has been found to be very sturdy and since it can survive even extreme events, this species can be used for farming and for restoration programs.

5.6 *Isochrysis galbana*

In the present study it was observed that the *I.galbana* had retained its capacity to multiply during 32 to 34°C, from an ambient of 24°C. Kaplan *et al.* (1985) had observed that temperatures higher than 32°C, reduced the cell production capacity of this species. This partly agrees with the present study since there was good cell multiplication in 32 and 34°C. This indicates that sudden change to 32°C followed by return of ambient temperature retains the capacity of *Isochrysis* to multiply and that it is resilient to 32°C

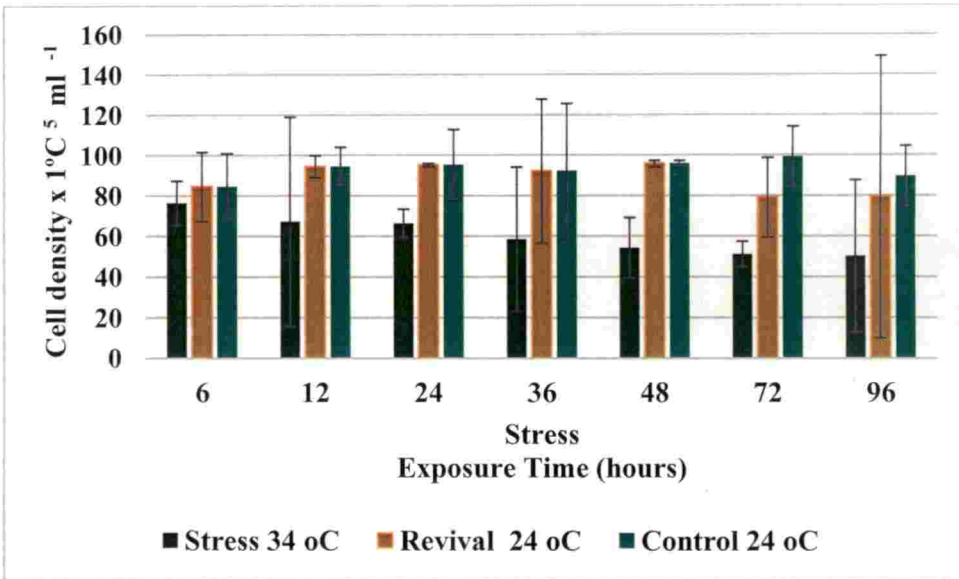


Fig 17: Cell densities of *Isochrysis galbana* on sudden exposure to temperature (32°C) stress and during revival in 24°C

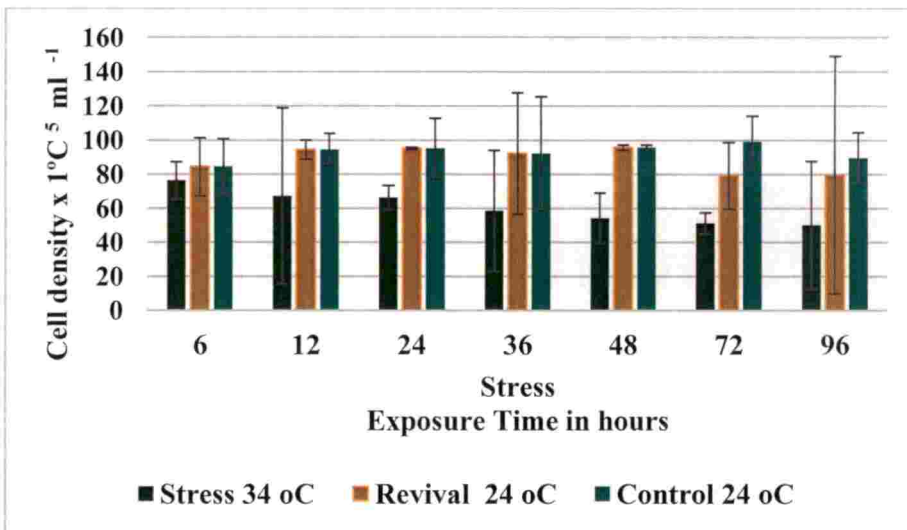


Fig 18: Cell densities of *Isochrysis galbana* on sudden exposure to temperature (34°C) stress and during revival in 24°C

The cell density of *I. galbana* in 34°C indicated in the present study showed that during the first six hours was lower than the control. But, after that in 12 to 96 hours, the cell densities were much lower than the revival and the control cell densities.

Temperatures 36°C had drastic impact on the cell density similar to the results obtained at 34°C by Kaplan *et al.* (1986). They had found that the optimal temperature for achieving highest algal yield was 27°C and that temperatures lower than 19°C reduced algal yield markedly. Thomas *et al.* (1984) had suggested that temperatures 22 to 27 gave good yield of *Isochrysis*, and the results of the present study also indicated that lower temperatures are more suitable for this species. All these observations clearly point to the fact that this species is vulnerable to extreme events, especially drought like situations.

Isochrysis has been extensively used as larval feed because of its high content of long chain polyunsaturated fatty acids (PUFAs) (Mai *et al.*, 1994). Several species of marine unicellular algae are the main feed for all growth stages of bivalves, and for various larvae of shrimps, crabs, lobsters and several species of finfishes which are widely farmed (Brown and Farmer, 1994). The biochemical composition of the algae is very crucial and if the quality is not good then the production in the hatchery comes down (Whyte *et al.*, 1989).

Research on live feed has shown that the quantity and quality of fatty acid of algal lipids are crucial to the growth, survival and development of various marine biota (De Pauw *et al.*, 1984; Koven *et al.*, 1989). Though in the present study the effect of temperature on the fatty acid profile of algae was not studied, literature indicates that temperature increase negatively impacts the quality of the phytoplankton used as feed. (Seto *et al.*, 1984; Mortensen *et al.*, 1988; Thompson *et al.*, 1992). In general, there is an inverse relationship between the percentage of PUFAs in lipid of the microalgae and temperature. So the threat of a poor quality of algae in higher temperatures, indicate that it may impact the recruitment success in natural water bodies.

Zhu *et al.* (1997) observed that temperature changes the content of protein and carbohydrate in the cultures of *Isochrysis* grown at 15 to 30°C and that highest protein content was found at the exponential growth phase at 15°C and the highest carbohydrate content was found at the stationary phase at the same culture

temperature. Here also the consequences of poor quality of live feed at higher temperature is indicated.

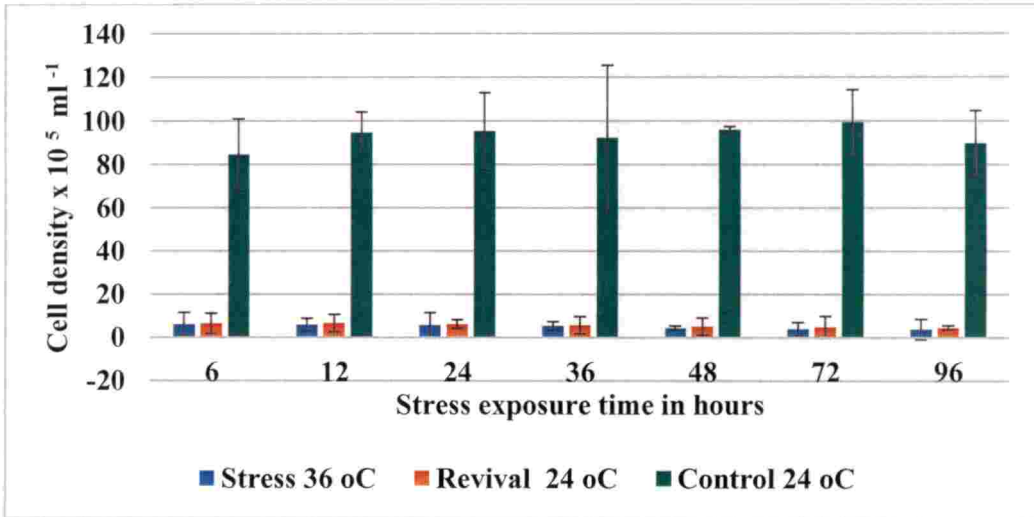


Fig 19: Cell densities of *Isochrysis galbana* on sudden exposure to temperature (36°C) stress and during revival in 24°C

Several studies conducted later in the present century also have observed that the biochemical composition and yield of micro algae are affected by increase in temperature. The relative content of unsaturated fatty acids especially trienoic fatty acids were found to vary in the cultures of *Chlorella vulgaris* and *Botryococcus braunii* (Sushchik, *et al.*, 2003). However, when the temperature increased there were no significant changes in the composition of extracellular unsaturated free fatty acids for these algae.

Similarly changes were observed in the cold phase also (McLarnon-Riches *et al.*, 1998). Chen *et al.* (2013) carried out extensive studies on effect of low temperature and the changes in the lipids of *Stephanodiscus* sp. They observed that the concentrations of both lyso-lipids and membrane lipids increased during the cold phase and recovery from the cold phase, respectively. Though in the present study,

the cold phase was not investigated, these studies indicated that such temperature changes can affect the quality of live feed.

Huang *et al.* (2017) report that high temperature of 35°C are observed in certain periods in China and that it affects the quality of *Isochrysis* production in the hatcheries. In China, the hatchery of many bivalve species like clams are operated during high-temperature seasons, when the water temperature in the maturation and seed production section frequently rises above 30°C and may even reach 35°.

Wu *et al.* (2017) observed that the docosahexaenoic acid (DHA) content increased in cultures grown at 20 and 35°C from 0 to 48 hour, with greater DHA accumulation in those cultures grown at 35 versus 20°C at the same time points. The results indicated that the content of DHA increased with the increasing temperature and that the nutritional value of this single cell algae in logarithmic phase improved with higher temperature. This study is optimistic and indicates that quality of *Isochrysis* will not go down at 35°C which is favourable to hatchery operations. Even though in the present study quality of *Isochrysis* was not tested, the multiplication of cells at par in higher temperatures up to 34°C, indicated its adaptive capacity. Hence it can be considered that higher temperature to a certain extent can be favourable to phytoplankton species like *Isochrysis galbana*.

5.7 Ocean acidification simulation – CO₂ based pH variations and impacts

Cladoceran species like *Daphnia salina* are considered as one of the best species which can be used as feed for feeding the larvae and early life stages of fin fishes bred in hatcheries. The advantages of this species is the fast growth and the low cost of production. Parra *et al.* (2016) have used a carbon dioxide injection system to vary the CO₂ levels and study its impacts on *Daphnia magna*. They have used pH 7 as the lower limit and in the twenty one day experiment under light and temperature-controlled conditions variations were observed in neonates production thus affecting population growth rates and secondary production. They have indicated that these changes can be related to energy allocation strategy and which can lead to ecological changes at higher tropic levels. In the present study at pH 7

there was no mortality and even the swimming behaviour was found to be same as in control, just as the observations of Parra *et al.* (2016). Smith (2016) in a detailed study on the impacts of carbon dioxide on observed that *Labidocera* spp. were one of the most sensitive copepods to high-CO₂ conditions and these were reduced by nearly 70% in abundance.

Apart from immobilization or mortality, reduced growth and or behavioural changes including swimming behavior of organisms are of ecological relevance as sub-lethal endpoints (Dodson *et al.*, 1995; Untersteiner *et al.*, 2005). In the present study, it was observed that the movement of cladocerans was affected by carbon dioxide variation in the sea water.

Observations by Perry *et al.* (2015) indicated that high partial pressure of carbon dioxide had no statistically significant effect on growth, survival, or otolith condition after 8 weeks of rearing of the juvenile scup, *Stenotomus chrysops*, a species that supports both important commercial and recreational fisheries. X-ray analysis showed that there was a slightly higher incidences of hyper-ossification in the vertebrae of some test fishes from the highest treatments compared to fish from the control treatments (Perry *et al.*, 2015).

Melzner *et al.* (2009) had stated that clownfish may be among the more resilient marine species to ocean acidification (OA) because of their ability for acid–base regulation. Studies have also indicated that some marine fish maybe more tolerant of OA due to exposure to natural fluctuations in ambient CO₂ in the habitats occupied by different life stages (Munday *et al.*, 2011; Hurst *et al.*, 2012). Moreover it has been observed that sensitivity during the early stages can be different and that even parental exposure to higher levels of carbon dioxide can influence the response of the life stages to elevated CO₂ levels ((Miller *et al.* 2012; Murray *et al.* 2014; Bignami *et al.*,2013).

Studies had shown completely different types of response by fishes to growth and survival to high CO₂ levels. Juvenile Atlantic cod was found to be affected when it was exposed to high pH levels. There was a reduction in weight but its survival

was not affected (Moran and Stottrup, 2011). In the present study survival was affected in lower pH, but above 6.8 it was not affected and the juvenile fishes accepted feed and showed normal swimming behaviour also.

In the present exposure experiments, in lower pH levels, 6.4 to 6.7 there was damage of caudal fin. Frommel *et al.* (2011) have observed that the Atlantic cod larvae exposed to increased levels of CO₂ had severe tissue damage in many internal organs, but larvae from the high treatment attained more weight than the control fish. Candelmo *et al.* (2013) found that fertilization was successful and survival from fertilized egg to hatch significantly increased with increasing CO₂ for winter flounder (*Pseudopleuronectes americanus*); however, their larvae were susceptible to sub-lethal effects. Frommel *et al.* (2013) observed that when the eggs and larvae of Baltic cod, *Gadus morhua* were exposed to high levels of CO₂, there were no effects on hatching, survival, development, and otolith size at any stage of development.

In the present experiments, pH levels of 6.8 and above did not affect the juveniles. In the eggs and larvae of the orange clown fish raised in seawater simulating CO₂ acidification scenarios predicted to occur in the next 50–100 years, Munday *et al.* (2009) did not find any detectable effect. In the early life stages of Atlantic herring (*Clupea harengus*) exposed to elevated pCO₂ revealed no effects on embryogenesis, hatch rate, total length, dry weight, and yolk (Franke and Clemmesen 2011). Similarly Munday *et al.*, (2015) did not observe any significant change in the survival and calcification of the common coral reef fish, the spiny damselfish *Acanthochromis polyacanthus*.

Hamilton *et al.* (2016) studied the species specific response of the rock fishes of the genus *Sebastes*. They observed that the copper rockfish (*Sebastes caurinus*) showed variation in behavioral lateralization, its swimming speed was reduced, changed in the enzyme activity, and expression of regulatory genes at high pCO₂ exposure. In contrast to this, the blue rockfish (*S. mystinus*), did not show significant changes in behaviour and swimming physiology. However, significant changes in



the expression of muscle structural genes as a function of pCO₂, was observed which can be considered as an indication of acclimatization potential. In the present study, both cladoceran and juvenile fishes exhibited behavioural changes. The study by Hamilton *et al.* (2016) had inferred *that* the capacity to adapt to varying water chemistry varied from one species to another.

Copatti *et al.* (2011) studied the effect of dietary salt and water pH on silver catfish juveniles. It was observed that low pH affected the juveniles as observed in the present study. But Copatti *et al.* (2011) were able to rectify these through dietary manipulations. Taucher *et al.* (2017) have described the results of a long-term in situ mesocosm experiment conducted to identify the response of natural plankton communities in temperate waters (Gullmarfjord, Sweden) to high carbon dioxide concentrations. The observations made through a plankton imaging system revealed pronounced temporal changes in the size structure of the copepod community and noted a decrease in copepod biomass thereby affecting the structure of plankton community.

SUMMARY AND CONCLUSION

CHAPTER 6

SUMMARY AND CONCLUSION

Frequency of climate related extreme events are increasing and impacting the coastal and marine ecosystems, making significant reflections on the fishery and aquaculture operations in the area. To understand how the biota would react to short spells of temperature and salinity changes, the study was conducted. The major expected output of the study was management advisories for increasing the preparedness of aqua farmers to climate related extreme events and suggestions for abiotic stress management of selected natural resources.

The resilience capacity of six commercially important resources (juvenile/adult stage) was studied; juveniles of two commercially important finfishes, *Etroplus suratensis* (pearl spot) and *Trachinotus blochii* (silver pompano), post larvae of the shrimp: *Penaeus monodon* (tiger prawn), seed and adult of the bivalves, *Perna viridis* (green mussel) and *Villorita cyprinoids* (black clam).

Temperature and salinity are some of the main abiotic parameters affecting the survival of marine biota. These change as intensity of extreme events like floods and droughts. Three different types of short term experiments simulating probable ecological conditions during extreme events were conducted on selected test species to understand

- 1) response to sudden salinity stress and its revival
- 2) response to sudden temperature stress and revival
- 3) impact due to varied water pH.

Sudden exposures were made to nine salinities from 5 to 45 ppt and seven higher temperatures 30 to 42° C. The exposure period for all treatments were 6, 12, 24, 36, 48, 56, 72 and 96 hrs. The response during revival to ambient salinity or temperature after stress exposure was studied for 24 hours. Response in terms of capacity to increase cell density of phytoplankton *Isochrysis galbana* to temperature 32 to 36°C was studied. Altogether 27 sets experiments on temperature, salinity and

pH sets stress simulations) out (nine on temperature stress and eight on salinity and remaining pH experiments were carried out.

Carbon dioxide was used to change the pH and the experiments were conducted for short period of 8 hours. The experimental pH were between 6.02 to 8.0. The impacts of pH variations was studied on the juveniles of clown fish *Amphiprion percula* and a cladoceran *Daphnia salina*

Based on the percentage mortality in the first two experiments, they were categorised as Resilient (R), Moderately Resilient (MR), Moderately Vulnerable (MV) and Vulnerable (V), if the total percentage mortality was 0 – 25, 26 – 50, 51-75, and greater than 75 respectively during the study period.

The study found that the juvenile and adult and young of different species had varied resilience capacity. *Villorita cyprinoides* (adult and juvenile) and *Etroplus suratensis* (Pearl spot) showed high resilience to sudden exposure to extreme temperatures (40-42°C). Adult *Perna viridis* was found to be moderately vulnerable to sudden exposure to high temperatures.

Species such as *T. blochii*, *P. monodon* and *P. viridis* (juvenile) were found to be vulnerable to high temperature (40 to 42°C). Correlation analysis between different species and their exposure to varying salinities (5 to 45 ppt) showed that *V. cyprinoides* (adult and juvenile), *E. suratensis* and *P.monodon* were resilient to the hyper-saline conditions (35 to 45 ppt). *P. viridis* (adult) and *T. blochii* showed higher mortality rate in both the low-saline (5 to 20 ppt) and hyper-saline (40 to 45 ppt) conditions.

Correlation analysis showed significant relationship between varying salinities and survival rate of each species. Pearl spot and tiger shrimp, green mussel (juvenile) and silver pompano, black clam (juvenile) and pearl spot, black clam (juvenile) and tiger shrimp were observed to have positive correlation of 0.9 to 1 significance level this indicates their compatibility for integrated farming.

From the observations, only three species: *V. cyprinoides*, *E. suratensis* and *P.monodon* were found to be adaptive and resilient to the low-medium salinities

whereas vulnerable to the extreme saline conditions. Species like *P. viridis* and *T. blochii* were found to be highly vulnerable, tolerant to only a narrow range of salinity conditions (20-35 ppt).

Phytoplankton *I.galbana* could tolerate upto 34°C; were capable of multiplication of cells on revival. However, they were vulnerable to sudden exposure to 36°C.

Response to extreme events and stress

Heavy precipitation related extreme events can be tolerated only by juvenile pearl spot, black clam and post larvae of tiger shrimp. Juvenile silver pompano, seed and adult mussel will not be able to survive such extreme conditions. Drought like situations or sudden increase in temperature up to 36°C can be tolerated only by juvenile pearl spot and black clam. Juvenile silver pompano, post larvae of tiger shrimp and, seed and adult mussels are vulnerable to increase in SST and drought like situations. Temperature stress can also lead to fin rot diseases in pearl spot, sudden moulting in shrimp larvae and reduce the capacity for byssal thread formation in mussels which can lead to slippage from ropes in mussel farms Erratic swimming, gasping and starving were also responses to extreme temperature stress.

Experiments on carbon dioxide based pH variation indicated that lower pH (less than 6.2) can affect the survival of clown fish juvenile. In pH range of 6.40 to 6.70 their behaviour changed, they were disoriented and gasping. Their caudal fin was also damaged. In the range of pH 6.80 to 8.00 the young ones of clown fishes were active, but they were not affected.

In zooplankton, *Daphia salina*, exposure to pH from 6.4 to 6.7 even for few seconds, damaged the appendages and they were found to be gasping and disoriented, without swimming capabilities. However in pH 6.8 and above, they were found to be active.

6.1 Advisories to farmers

Based on the behavioural response and survival of the resources, management advisories for reducing crop loss can be developed for mariculture which would increase the preparedness of the farmers to face climate change.

The study indicated that, it is advisable for pearl spot farmers to avoid stocking fish seed during summer months. Farmers should take measures to reduce the farm water temperature by better water circulation and shades.

Mussel farmers are advised to stock the seed only after stabilization of salinity at 25 ppt and also to provide shades on the racks to avoid temperature stress.

Mussel farmers are advised to harvest the mussel farm within 12 hours if the salinity in the farm area drops to below 25 ppt. Similarly harvest should be planned to avoid crop loss if the salinity increases above 35 ppt and if the sea water temperature in the farm increases above 34°C.

In marine hatcheries, phytoplankton crashes can be expected in outdoor tanks when the temperature increases above 34°C, hence hatchery operators are advised to take necessary precaution to prevent sudden collapse of *I. galbana* culture during summer.

6.2 Natural resource management

Based on the results of the experiments, suggestions for natural resource management programs can also be developed to increase the resilience of the native biota. In estuarine areas, it is advisable to plant more mangroves which can protect natural resources during peak summer period. Reduced water flow and stagnation of water should be rectified to ensure continuous flow.



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**IMPACT OF CLIMATE STRESS ON SELECTED MARINE
BIOTA**

by

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ABSTRACT

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ABSTRACT

Resilience capacity based on response to short term (6 hrs to 4 days) exposures to 5 to 45 ppt salinities and temperature (30 to 42°C) followed by revival indicated that the clam *Villorita cyprinoides* (adult and juvenile) and the teleost fish *Etroplus suratensis* (pearl spot) had high resilience to sudden exposure to extreme temperatures (40-42°C). Adult green mussel, *Perna viridis* (adult green mussel) was found to be moderately vulnerable to the high temperatures. *Trachinotus blochii* (silver pompano), post larvae of the shrimp *Penaeus monodon* and seed of *Perna viridis* were found to be vulnerable to high temperature (40 to 42°C).

From the observations, only three species, *V. cyprinoides*, *E. suratensis* and *P. monodon* were found to be adaptive and resilient to the low-medium salinities, but vulnerable to the extreme saline conditions. *P. viridis* (Adult) and *T. blochii* had higher mortality in both low-saline (5-20 ppt) and hyper-saline (40-45 ppt) conditions. Phytoplankton *Isochrysis galbana* could tolerate upto 34°C and were capable of multiplication of cells in higher temperature. However, they were vulnerable to sudden exposure to 36°C.

In the experiments on varied pH, swimming was found to be disoriented in juvenile clown fish, *Amphiprion percula* and the zooplankton, *Daphnia salina* when pH ranged from 6.40 to 6.70. In pH 6.8 and above they were found to be active.

Heavy precipitation related to extreme events can be tolerated only by juvenile pearl spot, black clam and post larvae of tiger shrimp. Juvenile silver pompano, post larvae of tiger shrimp and, seed and adult mussels are vulnerable to increase in SST and drought like situations.

Based on the behavioural response and survival of the resources, management advisories for reducing crop loss can be developed for mariculture which would increase the preparedness of the farmers to face climate change. Fish farmers are advised to avoid stocking fish seed during summer months. Farmers should try to reduce the water temperature in the farm by providing provisions for water circulation and shades.

Mussel farmers are advised to stock the seed only after the farm salinity stabilizes at 25 ppt. They should harvest the mussel stock in the farm within 12 hrs when the salinity drops below 25 ppt or if the salinity increases above 35 ppt and also when the sea surface temperature (SST) in the farm increases above 34°C. In marine hatcheries, phytoplankton crashes can be expected in outdoor tanks when the salinity increases above 34°C, hence hatchery operators are advised to take necessary precaution to prevent sudden collapse during summer.

In estuarine areas, it is advisable to plant more mangroves which can protect natural resources during peak summer period. Reduced water flow and stagnation of water should be rectified to ensure continuous water flow which would otherwise exacerbate impacts of extreme events related to climate change.

Keywords: Adaptive, marine biota, extreme events, vulnerable, salinity and temperature

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