Studies on eco-biology, captive breeding and rearing of alligator pipefish, *Syngnathoides biaculeatus* (Bloch, 1785)

A Thesis submitted to

Goa University

for the award of the degree of

Doctor of Philosophy

in

Marine Sciences

by

Sushant V. Sanaye

M. F. Sc

Department of Marine Sciences

Goa University, Taleigao Plateau-403 206, Goa

September, 2017

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Statement

As required by the Univeristy ordinance OB-9A.9 (viii), I state that the present thesis entitled "**Studies on eco-biology, captive breeding and rearing of alligator pipefish, *Syngnathoides biaculeatus* (Bloch, 1785)**" is my original contribution and the same has not been submitted on any previous occasion. To the best of my knowledge the present study is the firstcomprehensive work of its kind from the work mentioned.

The literature related to the problem investigated has been cited. Due acknowledgement have been made wherever facilities and suggestions have been availed of.

Sushant V. Sanaye

Certificate

This is to certify that the thesis entitled "**Studies on eco-biology, captive breeding and rearing of alligator pipefish, *Syngnathoides biaculeatus* (Bloch, 1785)**", submitted by Mr. Sushant V. Sanaye for the award of Doctor of Philosophy in Marine Sciences is based on his original studies carried out by him under my supervision. The thesis or any part thereof has not been previously submitted for any degree or diploma in any Universities or Institutions.

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(Sushant V. Sanaye)

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Chapter 1
General Introduction

1.1.Background Information

Eco-biological studies focus on inter-relationships of various biological aspects among organisms and their environment such as age and growth, length-weight relationship and condition factor, natural diet, reproductive biology and also biochemical constituents. Parameters such as age and growth, length-weight relationship and condition factor are more valuable in describing the general life history of fish inhabiting in particular environment. Such biological indices are more valuable from the management viewpoint, when they can be compared with similar data obtained from other populations in the different geographical and environmental conditions (Carlander, 1969). Distribution, growth, reproduction, nutritional profile, migration and behavior of a species are largely influenced by food availability and preferred prey items (Pfeiler*et al*., 2000). Therefore, knowledge of natural diet of any fish has eco-biological importance; it also can be used for sustainable management and conservation of population (Watanabe *et al*., 2006; La Mesa *et al*., 2007; Sara and Sara, 2007; Kitsos *et al*., 2008). The biochemical constituents of any fish denote its nutritional and energy status. The changes in the biochemical composition of the fish is essential for understanding the metabolism of different populations for providing an estimation of the energy content and for understanding the biochemical circulation of elements (Shamsan, 2008). Many aspects of the reproductive behaviors are commonly used either to catch the fish easily or to protect them if they are unduly vulnerable. The great fluctuation in the abundance of fish due to failure of the young to survival can also be explained. It is essential to adopt suitable measures for conservation and propagation of a particular fish (Murugan *et al*., 2009).

Seahorses, pipefishes, pipehorses and sea dragons are members of the order Syngnathiformes. It includes more than 323 species under 64 genera. The order Syngnathiformes comprises of five families and 64 genera (Nelson, 2006; Kuiter, 2009; FishBase, 2014). The families classified under this order are, Aulostomidae (trumpet fishes), Centriscidae (shrimp fishes), Fistulariidae (flutemouths), Solenostomidae (ghost pipefishes) and Syngnathidae (pipefishes, seahorses and seadragons). The family Syngnathidae (‘Syn’ means grown together or fused; ‘gnathos’ means jaws) characterized by the presence of dermal plates covering the body, tufted or lobed gills, pore-like gill vent, absence of pelvic fins and lacks true-jaw teeth (Dawson, 1985) and represented by about 298 species in 56 genera (Kuiter, 2009). Like seahorses, pipefish are also the most abundant and diverse group of family Syngnathidae and derive the name from their peculiarly long and slender pipe like body.Pipefishes are mostly stick-like with head in the line with the body. Body is semi-flexible due to the presence of bony plates and rings. Jaws are fused; snout is long and tubular with a small mouth at the tip. Pipefishes range in size from a few centimeters to more than 65 cm (Dawson, 1985). Fins are rayed and variably present. Dorsal fin is the principal locomotory organ which aids in swimming and maneuvering. The paired pectoral fins helps in balancing and moving up and down. Ventral fins are absent and the anal fin is small or degenerated. Some species have a moderately sized caudal fin, if absent the tail is prehensile.

The alligator or double-ended pipefish, *Syngnathoides biaculeatus* (Bloch, 1785) is widely distributed throughout the tropical Indo-Pacific with records from seagrass habitats extending from the northern Red Sea and the east coast of Africa, eastward to Japan, Samoa, the Tonga Islands, Australia (Dawson, 1985). Although, *S. biaculeatus* is considered to be the most heavily exploited pipefish in Traditional Chinese Medicine (TCM), known as 'Hailong' in TCM and has a history of over 600 years (Shi *et al.*, 1993; Pogonoski *et al.*, 2002). There are few estimates of trade volume to corroborate use of pipefishes in TCM (Barrows *et al.*, 2009). Vincent (1996) reported trade volumes of 1600–16500 kg dried pipefishes year^{-1} to Taiwan over the period of 1983–1993, whereas Martin-Smith *et al.* (2003) put this figure in the range of 7500–21300 kg year^{-1} into Hong Kong during 1998–2002 with the possibility of trade occurring from tropical countries, particularly from India, Malaysia, Philippines and Thailand (Martin-Smith *et al.*, 2003; Martin-Smith and Vincent, 2006).

1.2. Review of Literature

Species abundance of Syngnathidae is probably the highest in temperate and sub-tropical waters, while the greatest diversity occurs in the sub-tropical/tropical Indo-Pacific region from where about 70% of the recognized species are recorded (Dawson, 1985; Kuiter, 2000; Kuiter, 2009). Pipefishes are cosmopolitan in distribution between 71°N and 56°S latitudes (Dawson, 1985). Recent reports (Fleischer *et al.*, 2007) revealed a northward shift in distribution by 15° latitude and attributed this to increase in the northern sea surface temperature. About 15 genera (29%) are represented in the Atlantic Ocean and 47 genera (92%) in the Indo-Pacific region. Among this 4 genera and 1 sub-genus are endemic to the Atlantic Ocean and 36 genera and nine sub-genera to the Indo-Pacific (Dawson, 1985). Most species inhabit estuarine and coastal marine areas, some in freshwaters and very few even in hyper saline environments (Simmons, 1957). About 37 species have the ability to inhabit both fresh and marine waters (Nelson, 2006). Breeding populations of pipefishes have also been reported from freshwater environments (Dawson, 1970). Demersal populations occur in depths of a few centimeters to more than 400 m and the planktonic fish occur hundreds of kilometers offshore and over depths of several thousands of meters (Dawson, 1985).

Pipefishes are common residents occupying the seagrass meadows or residing at the sediment-water interface forming an important component of ichthyofauna of the sub aquatic vegetated habitats in coastal and estuarine environments (Dawson, 1985; Howard and Koehn, 1985). Dead seagrasses and detached algae also serve as shelter and a way of transport to shallower or deeper waters (Dawson, 1982; Vincent, 1995a; Kuiter, 2009). Pipefishes are also reported from coral reefs, marshes, mangroves and from a variety of bottom types from sand to rock and boulders (Dawson, 1982; Monteiro *et al*., 2001; Cakic *et al*., 2002). Syngnathid fishes are less mobile and often found attached to seagrasses, seaweeds, corals and any submerged substratum with their prehensile tail (Foster and Vincent, 2004). Long range migration observed in some pipefish species (Bayer, 1980; Lazzari and Able, 1990), while some species are found tostay in the shallow seagrass beds throughout the year (Howard and Koehm, 1985) exhibiting no seasonal movement.

Pipefishes are relatively inactive predators mostly using either a stationary ambush or a slower approach to capture their prey (Foster and Vincent, 2004). These fishes are characterized by pivot feeding (de Lussaanet and Muller, 2007; Wassenbergh *et al*., 2009). Being slow swimmers, they rely on crypsis for capturing the prey and escaping from predators. They are gape-limited, pipette-feeders with specialized feeding strategies (Howard and Koehn, 1985; Ryer and Orth, 1987) and feeding is confined during day-light hours (Ryer and Boehlert, 1983). Prey capture involves dorso-rotation of the head including mouth (Alexander, 1970; Muller and Osse, 1984) to rapidly approach the prey. The mouth cavity is expanded causing the suction of prey through the long tubular snout (de Lussanet and Muller, 2007). Micro-crustaceans such as copepods, amphipods and isopods form the dominant food item of pipefishes (Ryer and Orth, 1987; Franzoi *et al*., 1993; Dhanya, 2008; Kitsos *et al*., 2008).

In Syngnathid fishes, where males become pregnant and exhibit extreme degree of parental care, the developing embryos are carried out in specialized organ called brood pouch (Lockwood, 1867) located either in the trunk or tail region (Herald, 1959). In case of pipefishes, brood pouch varies in complexity. In few pipefishes, eggs are loosely attached to the ventral side of male and are completely unprotected. While in some pipefishes, eggs are placed in separate membranous compartment like pouch (Wilson *et al*., 2001). The functions of brood pouch vary with morphology of the pouch (Carcupino *et al*., 2002) from protection to aeration, osmoregulation and nourishment (Haresing and Shumway, 1981; Watanabe *et al*., 1997; Carcupino *et al*., 1997; 2002). Incubation period generally varies with species and abiotic factors and they give birth to relatively large young ones that start an independent life (Foster and Vincent, 2004).

Syngnathid fishes are not commonly consumed as food fish, but they acquire commercial importance as aquarium fishes and traditional medicine. Pipefishes are commonly represented in fabrics and artwork. They are sold dried or embedded in plastic and traded as souvenirs or curios (Dawson, 1985; Vincent, 1995a). Pipefishes are primarily sold for use in TCM serving same purposes as seahorses and are credited with curing ailments ranging from asthma and arteriosclerosis to impotence and urinary incontinence. Pipefishes are mainly used as kidney tonic and also provide remedies for skin ailments, high cholesterol levels, excess throat phlegm, goiters, lymph node disorders (Vincent, 1995a; Shi *et al*., 2006; Rosa *et al*., 2013) and clear toxins from the blood (Martin-Smith and Vincent, 2006). They are reputed to facilitate parturition (Vincent, 1995a). It is also considered an aphrodisiac; the larger the pipe fish, the more potent are its properties. Syngnathids were considered a natural remedy that was more efficacious than synthetic drug (Martin-Smith and Vincent, 2006). Along with pipehorses, the alligator pipefish *S. biaculeatus* alone is considered effective and pipefishes constitute about 35% of the contents in "Hailong tonic pills". Apart from the inclusion in TCM, pipefishes are also exploited for marine aquarium keeping for their peculiar shape and colouration. Among the pipefish species, banded pipefish, *Doryrhamphus dactyliophora* and the alligator pipefish, *S. biaculeatus* are most commonly traded. In Brazil, *Cosmocampus albirostris*, *Micrognathus* sp. and *Syngnathus* sp. were harvested for ornamental fish industry (Vincent, 1995a).

Syngnathids has been the subject of large scale target fishing which has resulted in rapid decline of the wild populations. This was worsened by indirect habitat destruction and loss (Vincent, 1995a). Substantial quantities of syngnathids are also trapped as by-catch in various fishing operations throughout the world. There are already reports on extinction of the riverine pipefish, *Syngnathus watermeyeri* in South Africa (Whitfield, 1996) and local extermination of *Doryichthys cuncalus* in India (Chhapgar and Pande, 1986). In Australia, *Vanacampus vercoi* was identified as vulnerable to habitat loss because of its restricted geographical range and preference to shallow waters (Pogonoski *et al.*, 2002). Considering the need for syngnathid conservation, all seahorses, 33 pipefishes, 2 seadragons and 5 pipehorses are listed in IUCN Red List of Threatened Animals (Version 3.1). Of the 33 listed pipefish species, 19 are classified as Least Concern, 10 are classified as Data Deficient, *Microphis deocata* as Near Threatened, *M. insularis* and *Vanacampus vercoi* as Vulnerable and *Syngnathus watermeyeri* as Critically Endangered. In India, all syngnathids are protected from capture and trade through the Wild Life Protection Act (WPA), 1972, and from 2001, pipefishes are listed under Schedule I of the WPA, 1972.

Knowledge over species diversity and distribution of syngnathid fishes is limited in Indian coastal waters. Till date, a syngnathid resource of Indian coastal waters comprises 10 species of seahorses and 13 species of pipefishes (Jones, 1969; GEC, 1996; Murugan *et al.*, 2008; Subburaman et al., 2014). These 13 species of pipefishes include, *Corythoichthys intestinalis intestinalis*, *Choeroichthys sculptus*, *Doryrhamphus melanopleura, Halicampus grayi, Hippichthys cyanospilos, H. spicifer, Ichthyocampus belcheri*, *Ichthyocampus carce, Microphis cancalus, Syngnathoides biaculeatus, Trachyrhamphus bicoarctatus, T. longirostris*, *T. serratus* (Jones, 1969; Murugan *et al.*, 2008).

The *S. biaculeatus* has a wide geographic distribution throughout theIndo-Pacific region (from the northern Red Sea, the eastern coast of Africa and eastward to Japan, Samoa and the Tonga islands) in seagrass meadows (Dawson, 1985), in which they are well camouflaged. It has been recorded near the islands of Micronesia and Samoa

(Randall *et al.*, 1996) and from Maumere Bay, Indonesia in seaweeds by Kuiter (2009). A study carried out by Murugan *et al.* (2008), suggested that *S. biaculeatus* is the most common pipefish in the Palk Bay and Gulf of Mannar regions (east coast of India). Bijukumar and Deepthi (2009) observed this species in trawl by-catch of Kerala state (west coast of India) in very low numbers. The TCM trade coupled with use as aquarium fish could threaten wild pipefish populations.

Currently this species is listed under the ‘Data Deficient’ category of IUCN red list (Bartnik *et al.*, 2008). A listing of Data Deficient does not imply that the taxon is not threatened but that not enough information exists to quantify or even estimate extinction risk and hence calls for extensive research for this species.

Information about *S. biaculeatus* was searched on internet search engines like Google, Google scholar. Databases such as FishBase (www.fishbase.org), sealifebase (www.sealifebase.org), encyclopedia of life (www.eol.org) were searched for gathering information for *S. biaculeatus*. ENVIS center on faunal diversity hosted by Zoological Survey of India, Kolkata was searched for alligator pipefish resources. Specimen depository at Zoological Survey of India (ZSI), Kolkata and Central Marine Fisheries Research Institute (CMFRI), Cochin was also visited for studying alligator pipefish collected from different parts of coastal India. Fish database, FishBase (www.fishbase.org) omitted into nine records of alligator pipefish, *S. biaculeatus*. Out of which, four specimens collected from Madras (Chennai), four specimens from Waltair and one specimen collected from Kerala state. Out of total nine, seven specimens were deposited in the Gulf coast Research Laboratory (GCRL) museum, University of southern Mississippi, (U.S.).

Museum records in Zoological Survey of India (ZSI), Kolkata having total ten preserved specimens in their head quarter and regional centers. These records confirms occurrence of *S. biaculeatus* from the coast of Travancore (now Kerala), Chennai and Andaman Islands. Museum records at ZSI, Kolkata have one specimen from east Island and six specimens from Nancowry Islands of Andaman. While Central Marine Fisheries Research Institute (CMFRI), Cochin museum have a specimen collected from Agathi, Lakshadweep Island. CMFRI museum also have other syngnathid fishes, some of which are not yet reported from coastal India. These include *Corythoichthys* *intestinalis intestinalis*, *Choeroichthys sculptus*, *Ichthyocampus belcheri*, *Doryrhamphus melanopleura* collected from Lakshadweep Islands.

Studies on *S. biaculeatus* are very limited (Sudarsan, 1966; Pogonoski *et al.*, 2002; Takahashi *et al.*, 2003; Dhanya *et al.*, 2005; Dhanya, 2008; Barrows *et al.*, 2009) and no detailed study is available on the biology of this species. Few studies dealing with reproductive behavior and life history of *S. biaculeatus* have been conducted and that too using wild caught pregnant males (Takahashi *et al.*, 2003; Dhanya *et al.*, 2005; Dhanya, 2008; Barrows *et al.*, 2009). Information on ecological parameters and captive rearing trials of this species is scanty except one study by Dhanya (2008).

It is expected that the results of this study would help in understanding biological aspects including its distribution along Indian coast, morphometrics, food and feeding habits, biochemical constituents and captive rearing of *S. biaculeatus*. Furthermore, captive rearing, reproductive behaviors and breeding would provide an alternative livelihood for the fisher folks who are dependent on the Syngnathids (seahorse and pipefish) catch from the wild in source countries as well as for its conservation through sea ranching programmes. Hence, the present study was taken up to address the following objectives.

1.3.Objectives

1. To generate baseline data about abundance, food and feeding, morphometric and meristic characters of alligator pipefish in natural habitat at selected locations.
2. To study reproductive behavior in captive condition.
3. To study captive rearing trials.

Chapter 2
Materials and Methods

2.1. Survey strategy

Surveys along the selected locations (Fig. 2.1) were conducted in coastal villages, fishing harbours and along creek areas with images and preserved specimens of alligator pipefish, *S. biaculeatus*.

2.2. Study locations

West coast of India

Gujarat

Survey was conducted along the coastal villages and fishing harbours at Mandvi, Vadinar, Porbandar, Veraval and Bhogat villages (Table 2.1).

Maharashtra

The survey carried out (Fig. 2.1) included villages namely Arnala, Versova, Colaba-Macchimar Nagar, Bhaucha Dhakka (Princes dock) in Thane district and Mumbai. Alibag, Revdanda, Murud, Shrivardhan were surveyed in Raigad district. Along Ratnagiri district, Harnai, Dabhol, Kalbadevi, Mirya, Sakhartar, Shirgaon, Karla, Nate, Jaitapur were surveyed, and along Sindhudurg coast, Vijaydurg, Devgad, Tambaldeg, Achara, Malvan, Vengurla Bandar, Shiroda were surveyed (Table 2.2).

Goa

An attempt was made to study the occurrence of these species along the coast by interacting with the fishermen community those are actively involved in regular fishing. Surveys at Teracol, Morjim, Shiolim, Malim jetty, Raibandar, Old Goa, Dona Paula, Odxel, Shirdona, Madkai, Cortalim, Chikalim, Kharaviwada, Kate-Bayana, Betul, Talpona, Kutbana jetty were conducted to assess the availability and earlier reporting of alligator pipefish (Fig. 2.1; Table 2.3). In addition, fish composition in the landings from beach seines and gill net operations in the estuarine embayment (where trawling

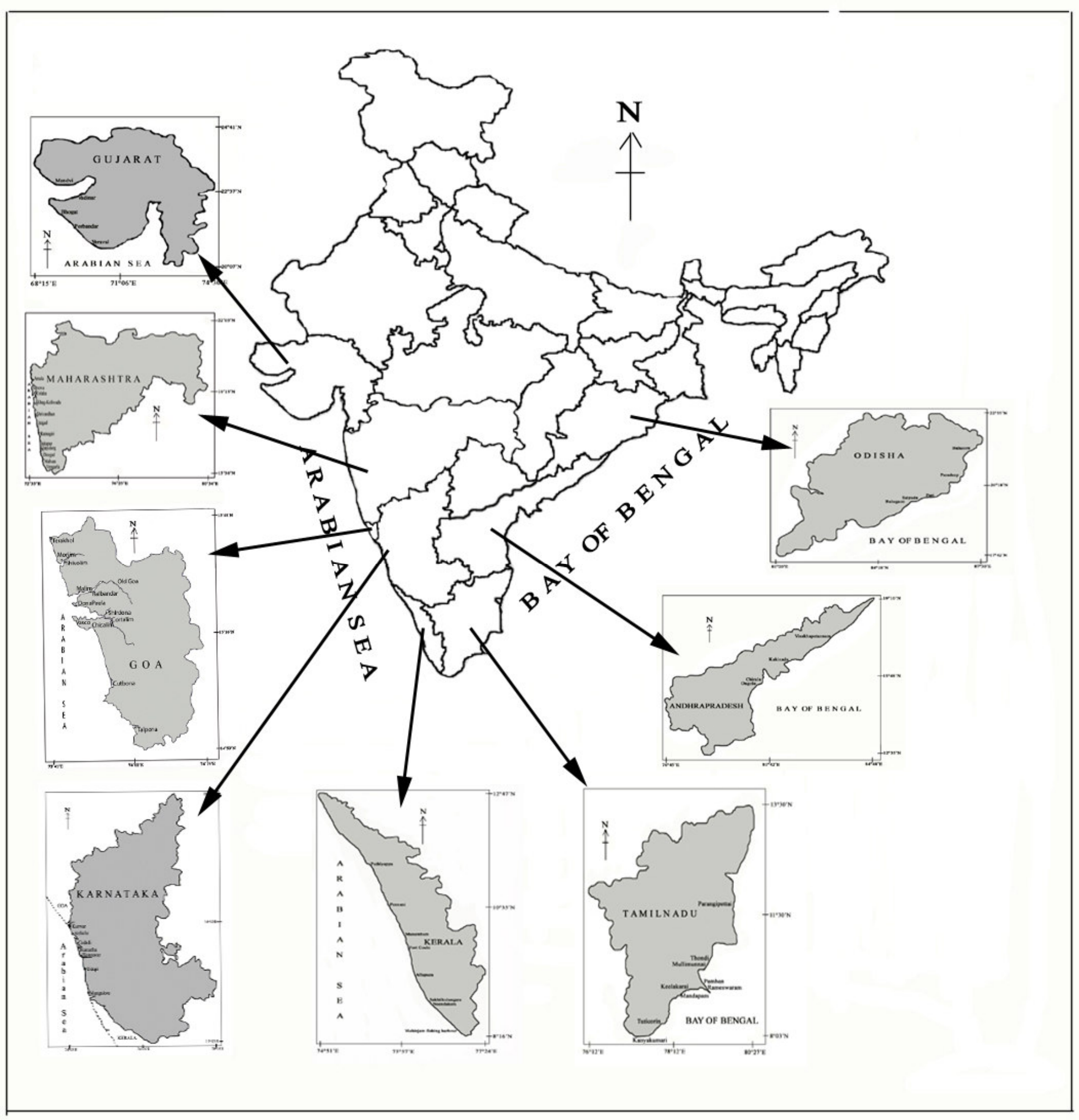

Fig.2.1. Map showing sample collection sites of *S. biaculeatus* along the Indian coast.

Table 2.1. Co-ordinates of places visited during field survey in Gujarat.

Sr. no.	**Place**	**Latitude**	**Longitude**
1	Mandvi port	22˚49'28.39" N	69˚21'04.85" E
2	Vadinar	22˚40'21.19" N	69˚72'14.55" E
3	Bhogat (Dwarka)	21˚58'46.40" N	69˚12'12.45" E
4	Porbandar	21˚38'31.89" N	69˚35'33.69" E
5	Veraval fishing jetty	20˚54'21.86" N	70˚23'01.01" E

Table 2.2. Co-ordinates of places visited during field survey in Maharashtra.

Sr. no.	Place	Latitude	Longitude
1	Arnala	19˚27'36.53" N	72˚44' 40.75" E
2	Versova	19˚08'35.65" N	72˚48'13.18" E
3	Colaba (Macchimar nagar)	18˚54'59.29" N	72˚49'21.19" E
4	Bhaucha Dhakka, Princes Dock	18˚57'29.56" N	72˚51'01.86" E
5	Alibag- Koliwada	18˚38'22.63" N	72˚52'56.74" E
6	Revdanda	18˚ 32' 36.42" N	72˚55' 52.66" E
7	Shrivardhan	18˚02'12.92" N	73˚01'07.86" E
8	Harne	17˚48'32.99" N	73˚05'23.18" E
9	Dabhol	17˚35'04.88" N	73˚10'36.63" E
10	Jaigad	17˚17'40.96" N	73˚13'18.98" E
11	Ratnagiri- Kalbadevi	17˚02'28.08" N	73˚16'51.58" E
12	Ratnagiri-Sakhartar	17˚02'29.08" N	73˚17'51.50" E
13	Ratnagiri-Shirgaon	17˚01'30.60" N	73˚17'27.35" E
14	Ratnagiri- Mirya	17˚ 01'42.38" N	73˚16'42.01" E
15	Ratnagiri-Mirkarwada	16˚59'58.83" N	73˚16'48.58" E
16	Ratnagiri-Karla	16˚58'35.59" N	73˚18'22.88" E
17	Jaitapur	16˚37'54.05" N	73˚21'37.88" E
18	Vijaydurg	16˚33'21.67" N	73˚20'15.75" E
19	Devgad	16˚22'45.06" N	73˚22'37.04" E
20	Devgad-Tambaldeg	16˚17'09.07" N	73˚24'42.91" E
21	Achara-Pirawadi	16˚12'00.09" N	73˚26'16.07" E
22	Malvan	16˚.31'6.09" N	73˚27'50.43" E
23	Vengurla Bandar	15˚51'26.31" N	73˚37'21.87" E
24	Vengurla- Shiroda	15˚45'27.82" N	73˚40'09.88" E

Table 2.3. Co-ordinates of places visited during field survey in Goa.

Sr. no.	Place	Latitude	Longitude
1	Terakhol	15˚43'06.82" N	73˚41'35.81" E
2	Morjim	15˚37'09.74" N	73˚44'15.96" E
3	Shivolim	15˚37'08.98" N	73˚45'59.10" E
4	Malim fishing jetty	15˚30'18.50" N	73˚50'01.61" E
5	Raibandar	15˚30'20.79" N	73˚51'50.93" E
6	Old Goa	15˚30'50.22" N	73˚48'09.73" E
7	Donapaula	15˚27'10.30" N	73˚51'50.93" E
8	Shirdona	15˚26'22.14" N	73˚51'38.22" E
9	Cacra Village	15˚27'05. 90" N	73˚50'32.85" E
10	Cortalim	15˚24'28.42" N	73˚54'35.37" E
11	Chikhalim	15˚24'15.16" N	73˚51'52.85" E
12	Vasco-Kharviwada	15˚24'02.10" N	73˚48'31.51" E
13	Vasco- Kate Bayna	15˚23'05.24" N	73˚48'28.83" E
14	Cutbona fishing jetty	15˚09'24.49" N	73˚57'13.87" E
15	Talpona	14˚59'00.44" N	74˚02'22.07" E

was restricted) was also recorded. Fish catch from the gill nets (25 mm mesh size) operated from fiber reinforced plastic (FRP) canoes fitted with outboard engine was observed on fortnightly basis. During the survey, along the coastal waters of Goa, central west coast of India, a single male alligator pipefish, *S. biaculeatus* (Bloch, 1785) accidentally caught in gill net with shredded branches of seaweed, *Sargassum* sp at depth of 20 m on 15 January, 2012 was recorded.

New record of *S. biaculeatus* along Goa coast

Material examined

One male specimen; SB-1, 177 mm total length, TL (location co-ordinates:15°25'42.20"N, 73°47'19.14"E; water depth: 20 m). S. Sanaye.

Comparative material

30 specimens, measuring 172-198 mm TL, were caught as by-catch in wind driven country trawl or '*Vallams*' mainly operated for crab/shrimp fishing in Palk Bay, Tamil Nadu (location co-ordinates: 9°39'24.48"N, 78°58'14.05"E and 9°46′35″N, 79°0′28″E; water depth: 5-10 m). S. Sanaye.

Karnataka

Places visited in Karnataka state (Fig 2.1) were Karwar, Ankola, Kumta, Honnavar, Murudeshwar, Kundapura, Malpe, Udupi and Manglooru (Table 2.4). Catch obtained by the FRP canoes and the bottom shrimp trawlers was observed and availability of syngnathid fishes was discussed with local fishermen.

Kerala

During field survey, fishing harbours at Neendakara, Sakthikulangara (Kollam district), Munambam (Ernakulam district), Fort Cochi, Alapuzza, Kayakulam, Ponnani (Malappuram district), Puthiyappa (Kozhikkode district) and Azheekkal (Kannur district) were visited (Fig. 2.1. and Table 2.5).

Table 2.4. Co-ordinates of places visited during field survey in Karnataka.

Sr. no.	Place	Latitude	Longitude
1	Karwar	14˚47'58.73" N	74˚06'43.71" E
2	Belekeri	14˚42'41.20" N	74˚15'51.50" E
3	Kumata	14˚25'11.73" N	74˚23'47.42" E
4	Honnavar	14˚16'33.14" N	74˚26'30.36" E
5	Murudeshwar	14˚05'38.42" N	74˚29'08.59" E
6	Kundapura	13˚38'17.96" N	74˚41'28.30" E
7	Malpe	13˚20'53.87" N	74˚41'58.07" E
8	Mangaluru	12˚51'21.44" N	74˚49'59.34" E

East coast of India

Tamil Nadu

Sites visited in the Palk Bay region were Mullimunnai, Thondi, Pamban and Parangipettai. Mullimunnai and Thondi village is fisher village in Ramanathapurum district of Tamil Nadu state (Fig. 2.1). These are one of the most productive and diverse fishing ground along the Tamil Nadu coast. It has good vegetation of seagrasses, seaweeds and sandy beaches. Therefore, a survey was conducted in coastal area of Mullimunnai and Thondi village and also discussed with local fishermen. Places visited in Gulf of Mannar region were Mandapam, Keelakarai, Tuticorin fishing harbor and Kanyakumari (Table 2.6).

Andhra Pradesh

Places visited in Andhra Pradesh (Fig. 2.1) were, Ongole, Chirala village, Visakhapattanam fishing harbor and Kakinada (Table 2.7). Survey was conducted at all the mentioned localities and discussed with local fishermen.

Odisha

Places visited in Odisha state (Fig. 2.1) were Chilika lagoon, Puri, Paradeep port, Balasore (Table 2.8). Five day survey was conducted at all mentioned localities and discussed with local fishermen.

2.3. Alligator pipefish, *S. biaculeatus* collection

A total of 331 dead and 40 live specimens of *S. biaculeatus* were collected from Tamil Nadu (Mullimunnai, Thondi, Mandapam and Tuticorin), Kerala (Neendakara and Sakhtikulangara) and Goa coast. After collection, the fishes were washed with ice cold distilled water and then immediately kept in ice box completely filled with ice and transferred back to field station at Mullimunnai/Thondi village, Tamil Nadu. Total length (TL) in cm and wet weight (g) were recorded for all individuals.

Table 2.5. Co-ordinates of places visited during field survey in Kerala.

Sr. no.	Place	Latitude	Longitude
1	Puthiyappa	11°19′ 07.43″N	75°44′47.72″E
2	Ponnani	10°46′ 55.95″N	75°55′05.60″E
3	Munambam	10°10′ 58.08″N	76°10′13.60″E
4	Fort Cochi	09˚58'04.80" N	76˚14'28.96" E
5	Alappuza	09˚28'19.37" N	76˚19'20.42" E
6	Kayamkulam-Azheekal	09˚07'53.36" N	76˚28'02.15" E
7	Sakhtikulangara	08°55′ 58.02″N	76°32′30.67″E
8	Vizhinjam fishing harbour	08°22′ 41.95″N	76°59′28.13″E
9	Neendakara	08°56′ 16.22″N	76°32′19.41″E

Table 2.6. Co-ordinates of places visited during field survey in Tamil Nadu.

Sr. no.	Place	Latitude	Longitude
1	Kanyakumari	08°04′51.59″N	77°33′08.73″E
2	Tuticorin	08°47′37.75″N	78°09′37.02″E
3	Keelakarai	09°13′37.57″N	78°47′07.17″E
4	Mandapam	09°16′ 36.74″N	79°09′04.35″E
5	Rameswaram	09°16′ 48.67″N	79°18′54.83″E
6	Pamban	09°16′ 37.76″N	79°12′17.12″E
7	Mullimunnai	09°39'24.48"N	78°58'14.05"E
8	Thondi	09°46′35.46″N	79°0′28.87″ E
9	Parangipettai	11°30′ 08.46″N	79°46′19.31″E

Table 2.7. Co-ordinates of places visited during field survey in Andhra Pradesh.

Sr. no.	Place	Latitude	Longitude
1	Ongole	15°26′ 18.39″N	80°10′45.83″E
2	Chirala	15°48′ 33.56″N	80°25′15.83″E
3	Kakinada	16°59′ 03.51″N	82°16′59.01″E
4	Visakhapatannam	17°41′ 48.56″N	83°18′02.22″E

Table 2.8. Co-ordinates of places visited during field survey in Odisha.

Sr. no.	Place	Latitude	Longitude
1	Chilika lake- Balugaon	19°44′ 35.96″N	85°12′45.32″E
2	Chilika lake- Satpada	19°39′ 55.49″N	85°26′14.29″E
3	Puri	19°48′ 08.37″N	85°51′05.50″E
4	Paradeep	20°17′ 17.62″N	86°42′14.54″E
5	Balaramgadi, Balasore	21°28′ 23.52″N	87°03′15.14″E

All live specimens were fed trice a day (0700, 1400 and 1800 hours) with mysids (*Mesopodopsis orientalis*), cultured adult *Artemia* (*Artemia salina*) and amphipods (*Grandidierella* sp.). Density of prey organisms were maintained @ of 200 – 300 nos.l^{-1}. Before every feeding, excreta and other waste from bottom of tanks were siphoned out and 20 – 30% water was exchanged with fresh sea water.

Systematic of *S. biaculeatus* (Bloch, 1785)

Kingdom: Animalia
Phylum: Chordata
Sub-phylum: Vertebrata
Infraphylum: Gnathostomata
Class: Actinopterygii
Subclass: Neopterygii
Infraclass: Teleostei
Order: Syngnathiformes
Suborder: Syngnathoidei
Family: Syngnathidae
Sub-family: Syngnathinae
Genus: *Syngnathoides*
Species: *Syngnathoides biaculeatus* (Plate 2.1)

2.4. General Methods

Morphometric measurements and Meristic counts

A total of 20 morphometric measurements (Table 2.9A) and 5 meristic counts (Table 2.9B)were studied following Cakic *et al.* (2002), Lourie (2003), Gurkan and Taskavak (2007) and Gurkan (2008) with species specific modification for *S. biaculeatus*. Line diagram of *S. biaculeatus* was drawn for better understanding of its body parts which were used in morphometric and meristic studies (Fig. 2.2).

Plate 2.1. Alligator pipefish, *Syngnathoides biaculeatus*.

Table 2.9A. Morphometricmeasurements and their description used in the present study

Sr. no.	Morphometric measurements	Description
1	Total length (TL)	Distance from the tip of the upper jaw to the end of tail tip.
2	Maximum body height (H)	Maximum distance between the dorsal and ventral surfaces of the body.
3	Maximum body width (iH)	Distance between the left and right side edges of the body at ventral surface in between 8^{th} and 9^{th} pre-anal rings.
4	Minimum body width (ih)	Distance between the left and right side edges of the body at dorsal surface in between 8^{th} and 9^{th} pre-anal rings.
5	Antedorsal distance (aD):	The distance from the end of head to the anterior point of insertion of the dorsal fin.
6	Postdorsal distance (pD) :	The distance from the insertion of the dorsal fin to the tip of the tail.
7	Length of dorsal fin base (lD)	Distance between the anterior and posterior points of insertion of the fin rays.
8	Height of dorsal fin (hD)	Distance between ventral points of insertion to the maximum length of fin rays.
9	Length of anal fin base (lA)	Distance between the dorsal and ventral points of insertion of the fin rays.
10	Height of anal fin (hA)	Distance between ventral points of insertion to the max length of fin rays.
11	Length of pectoral fin base (lP)	Distance between the dorsal and ventral points of insertion of the fin rays.
12	Head length (L_H)	Distance from the tip of the snout (upper jaw) to the point of gill operculum end.
13	Occipital height of head (HD)	The distance from the lowest point of the depression on the head immediately behind the coronet to the ventral surface of the body.

14	Head width (Hw)	Distance between right and left side at the head.
15	Snout length (SnL)	Distance from the tip of the snout (upper jaw) to the anterior side of the tubercle/spine immediately in front of the orbit (pre-orbital tubercle/spine).
16	Snout depth (SnD)	Minimum distance between the dorsal and ventral surfaces of the snout.
17	Mouth width (MW)	The minimum distance between the right and left sides of the snout.
18	Eye diameter (ED)	Distance between the anterior and posterior inside edges of the orbit (eye socket).
19	Post-orbital length (PO):	Distance between the posterior edge of the orbit to the anterior start of pectoral fin.
20	Inter-orbital distance (ID)	The shortest distance between the eyes.

Table 2.9B.Meristic counts and their description used in the present study

Sr. No.	Meristics counts	Description
1	Number of rays in dorsal fin (D)	Number of bony rods that support the fin membrane. The last ray sometimes appears double, but is counted as one because the two parts are joined at the base.
2	Number of rays in anal fin (A)	The number of bony rods that support the fin membrane.
3	Number of rays in pectoral fin (P)	The number of bony rods that support the fin membrane.
4	Number of Preanal rings (PaR)	The number of external raised bony rings that encircle the body from just behind head to at the end of anus.
5	Number of postanal rings (PoAR)	The number of external raised bony rings that encircle after anus end towards the tail tip. The rings become indistinct and cracks appear on the ventral surface between the rings.

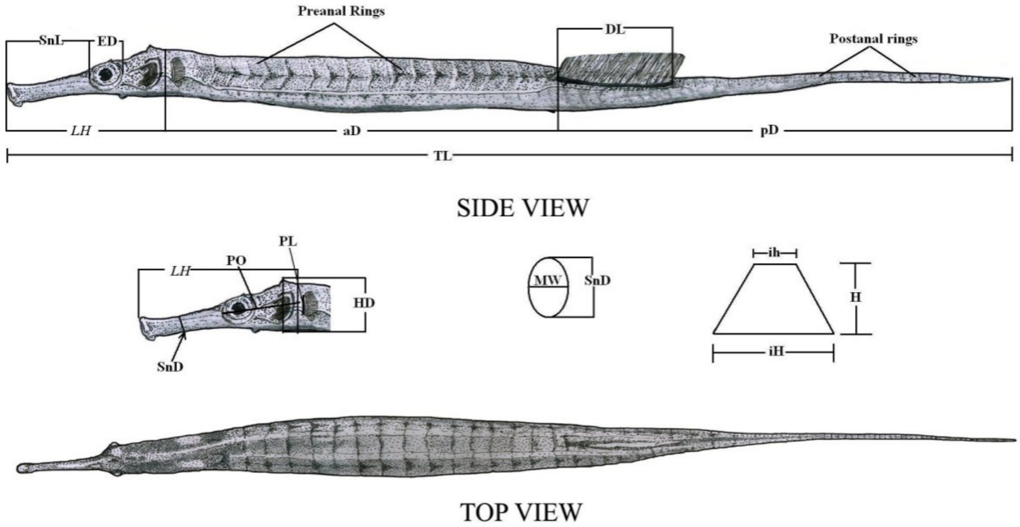

Fig. 2.2. Line diagramme of *S. biaculeatus* showing side and dorsal view.

SnL: Snout length; LH: Head Length; OD: Orbital Diameter; TL: Total Length; DL: Dorsal fin base length; SnD: Snout Depth; PL: Pectoral fin base length; HD: Head Depth; PO: Post orbital distance; aD: Anterodorsal Distance; pD: Postdorsal Distance; MW: Mouth Width; H: Body Height; iH: Maximum body width; ih: Minimum body width.

Females were identified by the presence of a white zigzag pattern on the ventral side of abdomen and accompanied by 15-20 blue dots, often interspersed with red patches (Takashi *et al.*, 2003; Barrows *et al.*, 2009). Distinguishing between non-pregnant males and females for individuals of less than 155 mm was often not possible, and in such cases the individual was recorded as juvenile (Barrows *et al.*, 2009). Morphometric measurements and meristic counts obtained from single specimen from Goa and 18 specimens from Kerala were not included in statistical analysis due to less sample size but were presented in the respective tables.

Length-weight relationship and Fulton's condition factor

Total length (TLin cm) and total wet weight (W in g) were measured from alligator pipefishes (Gurkan, 2008). The length–weight relationship was established using the formula,

$$\mathbf{W = aTL^{b}}$$

Using linear regression analysis, **log TL = a + b log W**

Where a = intercept of the regression curve and b = regression coefficient.

Due to additional weight of incubating eggs, pregnant males were not included in length-weight analysis. The regression line was computed using the method of simple least-square regression analysis.

The Fulton's condition factor (*K*), which determines the physical and environmental condition of the fish and is used for comparing the condition, fatness or well-being of fish. The Fulton’s condition factor (*K*) was calculated from equation (Ricker, 1975; Cakic *et al.*, 2002).

$$\boldsymbol{K} = \mathbf{1000\ W\ (TL)^{-3}}$$

Where *K* = condition factor, W = weight of fish (g) and TL = total length of fish (cm).

Natural Diet composition

Gut removal

A total of 56 specimens of *S. biaculeatus* were used for diet composition studies. After length weight measurements, *S. biaculeatus* individuals were dissected by incision at ventral surface (Plate 2.2) and gut was removed. Gut length and weight were recorded with the help of calibrated ruler (0.5 mm accuracy) and with the help of digital balance (accuracy 0.05 mg), respectively. Gut fullness was estimated visually and by measuring portion of the filled gut with ruler, on a six point percentage scale: empty (0%), moderately full (12%), ¼ full (25%), half full (50%), full (75%) and very full (100%). All the guts were transferred in 10% buffered formalin with appropriate labels and brought back to the laboratory at National Institute of Oceanography, Goa. At the laboratory, all the guts were cut opened by length wise incision and the gut content were washed through 125 µm mesh and all the prey individuals were counted with the help of counting chamber under stereoscopic microscope (Olympus SZX7, Japan) and identified to the possible taxonomic levels. Broken individuals of prey were identified up to group levels and listed as unidentified. Gape size of *S. biaculeatus* was shown in Plate 2.3.

Gut content analysis

Data collected from gut content analysis were used to calculate following indices. Methods and formula as described in earlier published literature (Hyslop, 1980; Williams, 1981; Kelleher *et al.*, 2000; Woods, 2002; Murugan, 2004; Kitsos *et al.*, 2008) were used.

$$\textbf{Frequency of Occurrence } (\%F_0) = n100N_s^{-1}$$

Where, n = the number of guts containing certain prey

N_s = the number of total guts examined

$$\textbf{Percentage of prey } (\%N) = n'100N_p^{-1}$$

Where, n'=the total number of individuals of a certain prey

N_p = the total number of prey items.

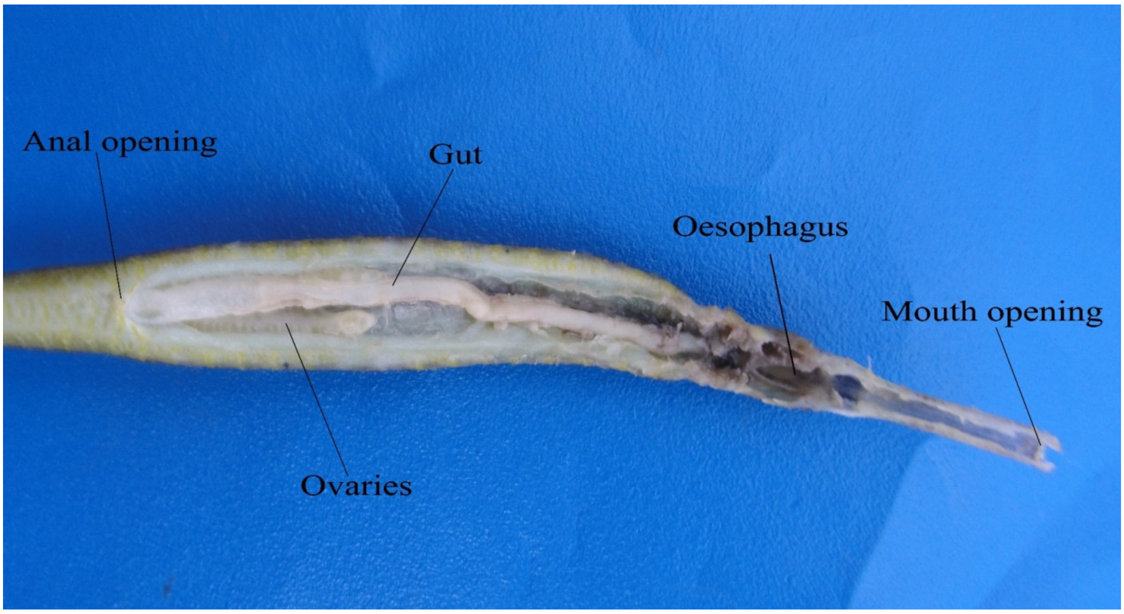

Plate 2.2.Ventrally opened *S. biaculeatus* showing gut and other parts.

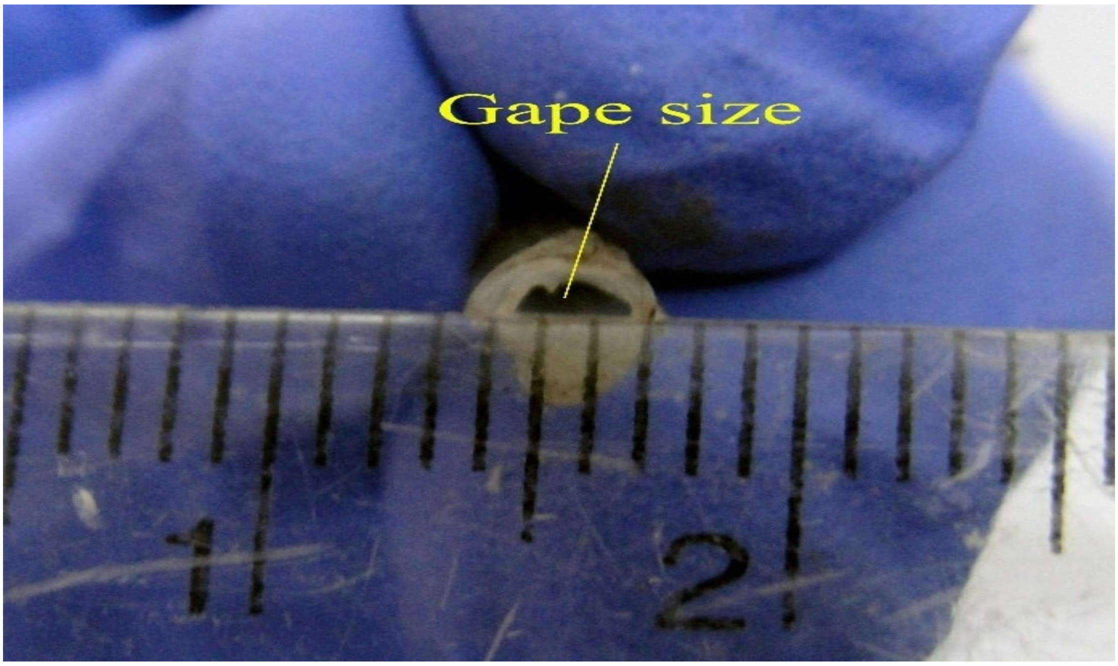

Plate 2.3. Gape or mouth opening of *S. biaculeatus.*

Based on the number of empty guts, the vacuity coefficient index (I_v) was calculated

$$\textbf{Vacuity coefficient index } (I\text{v}) = E_v 100 N_s^{-1}$$

Where, E_v = the number of empty guts

N_s = the number of total guts examined

Gastro-Somatic Index (GSI) in % was calculated to find out the feeding intensity by applying the following formula. Data presented as mean ± SD.

% GSI = weight of gut / weight of fish X 100

Relative gut length which is ratio of gut length to the total length (TL) of alligator pipefishes was calculated.

Relative gut length = Length of gut / total length of fish

Biochemical composition

A total of 40 specimens of *S. biaculeatus* were served as material for the present study. Mean total length (TL) and wet weight (g) of the fishes used for biochemical studies were 18.5 ± 3.51 cm and 5.45 ± 0.57 g, respectively. After collection, the fishes were washed with ice cold distilled water and then immediately frozen in liquid nitrogen and preserved at –80 °C until analyses were carried out. Lyophilised and powdered alligator pipefishes were used for the preparation of extracts and chemical analysis. Chemical analyses were performed in duplicates while total phenolic content and other antioxidant properties were performed in triplicate.

Proximate composition

Proximate composition of alligator pipefish were analyzed by following the standard methods described in AOAC (2005). Moisture was determined by drying the wet samples at 105 ºC for 24 hrs to a constant weight in hot air oven (Biotechnics India, Mumbai, India). Ash was estimated by incinerating samples in muffle furnace (Biotechnics India, Mumbai, India) at 600 ºC for 6 hours. Nitrogen content of samples was estimated by using micro-Kjeldahl acid digestion method. Crude protein was calculated by multiplying total nitrogen content by 6.25. Crude lipid was estimated by using Soxhlet extraction apparatus using petroleum ether as solvent. The nitrogen free extracts (carbohydrates, vitamins and other non-nitrogen soluble compounds) was computed by remainder method (Woods and Aurand, 1977).

Fatty acid profile

Total lipids were extracted by homogenizing the lyophilised powdered samples in five columns of chloroform/methanol (2:1, v/v) and run according to the method of Folch *et al.* (1957). The lipids were converted into fatty acid methyl esters (FAMEs) then identified by gas chromatography after re-dissolvig in hexane. The FAMEs were analyzed using a Shimadzu GC–Mass Spectrometer, QP-2010 Ultra EI & PCI (Shimadzu, Japan). FAMEs were separated on a CHROMPACK (Bristol, PA, USA) WCOT 25 × 0.25 mm ID, 0.2 μm film thickness capillary column using a temperature program from 160 ºC up to 235 ºC with an increase rate of 1.5ºC min^{-1}. Initial and final time was 0 and 15 min, and total run time was 50 min. The injector temperature was 260 ºC, FID temperature 260 ºC and helium gas was used as the carrier gas. Identification of fatty acids in the samples was performed by comparison with chromatograms of fatty acids standard (C_4 – C_{24} fatty acids) from Sigma Aldrich, India. Peaks in the chromatograms were identified by comparison with retention times and peak areas of FAMEs standards. Fatty acids composition (%) was calculated from the total identified fatty acids and values are presented as % mean ± standard deviation.

Amino acid profile

Amino acid composition of alligator pipefish sample was analyzed with Waters AccQ•Tag™ amino acid analysis method. Amino acid composition was determined after acid hydrolysis of powdered sample (50 mg) in 6 N HCl for 24 h at 110 ºC, dried hydrolysate was again re-suspended in 100 ml of ultrapure H_2O. 10 μl of above solution was added to 90 μl of reaction buffer (AccQ• Fluor Borate Buffer, Waters, Milford, USA) to make 100 μl solution. From this 10 μl solutions were then injected into column (Waters AccQ• Tag™ amino acid analysis column). Separation of different amino acids was carried out with HPLC (Waters Corporation amino acid analyzer, Milford, USA). Cystine and tryptophan could not be detected after acid hydrolysis. Asparagine is determined as aspartic acid and glutamine as glutamic acid. Individual amino acids were analyzed by comparing their retention time with those of amino acid standards carried out under identical conditions and expressed as percentage of total amino acids.

Trace element analysis

Lyophilised and powdered sample (5 g) was used for determining the concentration of trace elements in duplicate. A microwave accelerated digestion system (CEM-MARS 5) was used to digest a wide variety of trace metals in the laboratory. This system condenses materials of different matrices, allowing for the analysis of volatile metals, such as Hg. During the digestion portion of the Hg analysis, 1 ml of HNO_3 and 3 ml of HCl were added to 5 g of sample, and the volume was increased to 10 ml using Milli-Q water. Teflon vessels containing the samples were kept in the double walled, outer liner of the digestion bomb, capped with a sensor head and pressure rupture disc. Sealed vessels were then placed in the microwave carousels in the same manner as for digestion. Each set of samples was accompanied by a blank, spike and certified reference material. Trace metals were analyzed using Graphite Furnace Atomic Absorption Spectrometry (GF-AAS, PerkinElmer, Analyst 600) and an Inductively Coupled Plasma Optical Emission Spectrophotometer (ICP-OES, Optima 7300 DV, Perkin Elmer, Inc., Shelton, USA). The precision and accuracy of analysis were verified by replicate measurements ($N = 5$) of target metals in a standard reference material of marine biota sample (TORT-3 Lobster hepatopancreas reference material for trace metals; National Research Council, Canada). The analysed values obtained for the reference materials were found to be in good agreement with the certified values.

Carbon:Nitrogen (CN) ratio

Amount of carbon and nitrogen was analyzed with help of NC organic elemental analyser (FLASH 2000, Thermo Scientific, India). Lyophilised and powdered *S. biaculeatus* were dried in oven at 105 ºC for one hour and kept for cooling for absorbing their natural moisture and then taken for CN analysis. About 300 mg of sample was taken into tin container and kept in auto sampler of NC organic elemental analyzer. Samples were run in duplicate and levels of CN were measured.

Total phenolic content and Antioxidant activities

Chemicals

Folin-Ciocalteu reagent, sodium carbonate, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), potassium ferricyanide, trichloroacetic acid (TCA), ethylenediamine tetracetic acid (EDTA) disodium salt, ferric chloride, ascorbic acid (ASA), hydrogen peroxide, potassium chloride, ferrous chloride, ferrous sulfate, ferrozine, 2-deoxy-D-Ribose were procured from Sigma Aldrich, India, while methanol and hydrochloric acid (HCl) were of analytical grade from Merck, India.

Preparation of extract

The freeze dried pipefishes were grounded to a fine powder and passed through 0.5 mm mesh sieve. 5 g of the powder were mixed with 100 ml of concentrated HPLC grade methanol (w/v), incubated in a platform shaker (Remi Orbital shaking incubator Model: RIS - 24 BL, India) for 24 hours at 180 rpm at room temperature (28 °C). The mixture was centrifuged at 3500 rpm for 10 min at 4 °C and filtered with Whatman No. 1 filter paper. Methanol in the extract was removed by rotary evaporation (BUCHI Rotavapor R-200) at 45 °C. Each extraction was conducted in duplicate. The extraction yield was expressed as percentage of dried sample. The dried duplicate extracts were pooled together and stored at –80 °C until analyzed. Dried extract was then re-dissolved in methanol (HPLC grade) at a concentration of 2 mg ml^{-1} as a stock extract solution. The stock extract solution was used for the determination of antioxidant activities. Three different extract concentrations (200, 400 and 800 µg) were tested in triplicate. Values of different standards used during TPC and antioxidant activities evaluation are shown in Table 2.10.

Total Phenolic content (TPC)

Total phenolic content (TPC) was quantified following the method suggested by Slinkard & Singleton, (1977). The reaction mixture contained 0.1, 0.2 and 0.4 ml of sample extract; 1 ml of Folin-Ciocalteu reagent (1:5 dilutions swirl to mix with distilled water and incubated for 1 - 8 min at room temperature (28 °C) were added and mixed thoroughly. 3 ml of 2 % sodium carbonate solution was added to the mixture and was allowed to stand for 2 hours, with intermittent shaking. The absorbance was measured on spectrophotometer (UV 1800, Shimadzu, Japan) at 760 nm, and compared with a standard curve of gallic acid (10 ~100 µg ml^{-1}). The amount of TPC is expressed as mg gallic acid g^{-1} dried extract.

Reducing power

Reducing power (RP) of *S. biaculeatus* extracts was determined as suggested by Oyaizu (1986). Reaction mixtures were prepared by adding 2.5 ml of phosphate buffer (0.2 M, pH 6.6), 2.5 ml potassium ferricyanide (1%) and pipefish extracts (0.1, 0.2 and 0.4 ml). Reaction mixtures were incubated at 50 °C in water bath for 30 min and allowed to cool at room temperature (28 °C). To each reaction mixture, 2.5 ml of 10 % TCA was added and centrifuged at 2000 rpm for 10 min. The supernatant (2.5 ml) was added with 2.5 ml of distilled water and 0.5 ml ferric chloride (1.0 %), allowed to react for 10 min at room temperature (28 °C) and then absorbance was measured at 700 nm. Ascorbic acid (ASA) solution (100 µg ml^{-1}) was used as standard. Absorbance values of reaction mixture are directly proportional to the levels of reducing power.

Table 2.10. Values of standards at different concentrations used in TPC and antioxidant activity.

Standard concentrations (µg)	Reducing power (Absorbance)	Metal chelating activity (%)	DPPH radical scavenging activity (%)	Hydroxyl radical scavenging activity (%)	LPX inhibition (%)
10	0.29±0.009	41.77± 0.06	38.86±0.17	90.43±0.14	85.77 ±0.004
20	0.41±0.002	82.42±0.02	43.43±0.05	92.88±0.18	89.08±0.01
40	0.63±0.001	87.62±0.12	45.54±0.021	93.02±0.009	96.12±0.15
80	1.08±0.15	97.26±0.10	52.27 ±0.013	93.19±0.10	98.05±0.008
100	1.28 ±0.01	98.71±0.03	64.83±0.08	96.92±0.08	98.65±0.01

Ascorbic acid was used as standard for Reducing power, Hydroxyl radical scavenging activity and LPX inhibition; while EDTA was used for metal chelating activity and BHT was used for DPPH radical scavenging activity.

Metal chelating activity

Method suggested by Dinis *et al*. (1994) was used for measuring the metal chelating activity. Briefly, different volumes of the extract (0.1, 0.2 and 0.4 ml) were added to 0.2 ml of 2 mM ferrous chloride. The reaction mixture was initiated by addition of 0.2 ml of ferrozine. The mixture was shaken vigorously and allowed to stand for 10 min at room temperature (28 °C). The absorbance was measured at 562 nm using spectrophotometer. The metals chelating activity of the extract was compared with the standard EDTA (100 µg ml^{-1}). Reaction mixtures without ferrous chloride and ferrozine or complex formation molecules were served as control. Metal chelating activity (%) of pipefish extract was expressed as follows:

$$\mathbf{MCA\ (\%) = [A_0 - A1/A_0] \times 100,}$$

Where, A_0 — absorbance of the control and A_1 — absorbance of the test compound.

DPPH radical scavenging Activity

The free radical scavenging potential in alligator pipefish methanol extract was measured following the method suggested by Blois (1958) using 2, 2-diphenyl-1-picrylhydrazyl (DPPH). The initial absorbance, and absorbance after 30 min of the reaction mixture [incubated in dark; 2.5 ml of DPPH solution (0.1 mM in methanol)] and sample extract (0.1-0.4 ml) was measured at 517 nm. Butylated hydroxytoluene (BHT, 100 µg ml^{-1}) was used as standard. The DPPH radical scavenging activity (%) was calculated using the formula:

$$\mathbf{DPPH\ radical\ scavenging\ activity\ (\%) = [A_0 - A_1 / A_0] \times 100,}$$

Where, A_0 — is the initial absorbance (0 min) and A_1 — is the final absorbance (after 30 min)

Ferric Reducing Antioxidant Power (FRAP)

The total antioxidant capacity of alligator pipefish extract was determined by FRAP assay, which depend upon the reduction of ferric tripyridyltriazine (Fe^{+3}— TPTZ) complex to the ferrous tripyridyltriazine (Fe^{+2}— TPTZ) by a reductant at low pH.

Method described by Benzie *et al*. (1996) was used to perform FRAP assay. For the determination of FRAP activity.Working solution was prepared using 2.5 ml acetate buffer (300 mM, pH 3.7), 2.5 ml TPTZ solution (10 mM 2,4,6-tripyridyl-striazine (TPTZ) in 40 mM HCl.) and 2.5 ml (20 mM) ferrous chloride solution. Five different concentrations of ASA (100~1000 µg ml^{-1}) were used as standard. FRAP assay was done by mixing 1.5 ml freshly prepared working solution and different sample concentration. The mixture was incubated for 10 min and then intensity of blue coloured complex was recorded at 593 nm. The results were expressed as mg ASA g^{-1} dried extract.

Lipid Peroxidation (LPX) inhibition assay

In-vitro lipid peroxidation inhibition assay was performed by the method described by Jena *et al*. (2010). Fresh sheep liver was obtained from local slaughter house, washed with ice cold potassium chloride (1.15 %) and homogenized (10 % w/v) with Teflon Potter-Elvejhem homogenizer. Homogenate was filtered through cheese cloth and centrifuged at 10000 rpm for 10 min, at 4 °C. Supernatant was used for LPX assay.

Peroxidation of liver homogenate was induced by ferrous sulfate solution. Liver homogenate was incubated with 100 mM of ferrous sulfate for 30 min at 37 °C; the formation of thiobarbituric acid reactive substances (TBARS) in the incubation mixture was measured at 532 nm with ascorbic acid (100 µg ml^{-1}) as standard. Reaction mixture without any extract and standard was used as control. The LPX inhibition (%) was calculated as

$$\textbf{LPX inhibition (\%)} = [1 - (A_0 - A_1 / A_2)] \times 100,$$

Where, A_0 is the absorbance in the presence of extract, A_1 — absorbance without sheep liver homogenate and A_2 — absorbance of the control (without extract or standard).

Hydroxyl radical scavenging (HRS) activity

The ability of the extract to inhibit hydroxyl radical-mediated 2-deoxy-D-ribose degradation was determined spectrophotometrically by the method described by Chung *et al*. (1997). The reaction mixture containing 0.2 ml of ferrous sulphate (10 mM), 0.2 ml of EDTA (10 mM), 0.2 ml of 2-deoxy-D-ribose (10 mM), 0.1-0.4 ml of extract sample and 1 ml of phosphate buffer solution (0.2 M, pH 7.4) was mixed. Then 0.2 ml of hydrogen peroxide (10 mM) was added to the reaction mixture and incubated at 37 °C for 4 hours. Thereafter, 1 ml of 2.8 % TCA and 1 ml of 1 % TBA were added to the tubes. The samples were mixed and heated in a waterbath at 100 °C for 15 min. The mixture was cooled by immersion for 5 min in an ice. The absorbance was read at 532 nm with ascorbic acid (100 µg ml^{-1}) as standard. Reaction mixture without hydrogen peroxide served as control. The inhibition of deoxyribose degradation was calculated using below equation

$$\textbf{HRS activity (\%)} = [A_0 - A_s/A_0] \times 100,$$

Where A_0 absorbance of the control, A_s absorbance of the sample.

Captive breeding and rearing studies

Sexual determination (Plate 2.4.) based on white zigzag pattern present in females at ventral abdominal part, as reported in Takahashi *et al.* (2003) was carried out. Live specimens collected from Mullimunnai village were then packed in polythene bags with sea water and filled with pure oxygen and tightly tied with rubber bands. All specimens were transported to Aquaculture laboratory at CSIR-National Institute of Oceanography, Goa. All specimens were alive and then acclimatized to the laboratory conditions. Mean total length (TL) and wet weight (g) of collected specimens were 19.6 ± 1.5 cm and 5.41 ± 0.61 g for males and 19.1 ± 1.1 cm and 6.40 ± 0.46 g for female, respectively. Alligator pipefishes were maintained in 1000 liter FRP tanks and rope mesh (4 mm diameter) was provided as holdfast. Optimum water quality conditions were maintained in the rearing tanks (salinity 28 ± 2 ppt, water temp 27 ± 2 °C, dissolved oxygen > 6 mg l^{-1}, pH 7.6 ± 0.3, NO_2–N < 0.02 mg l^{-1} and NH_3/NH_4 - 0 mg l^{-1}).

Live feed management

Mysids, *Mesopodopsis orientalis*

Mysids, *Mesopodopsis orientalis* (Plate 2.5) were collected from brackish water fish farm, Directorate of Fisheries, Govt. of Goa located at Old Goa, Goa (15˚30'50.22"

Plate 2.4.Male and female specimens of *S. biaculeatus*.

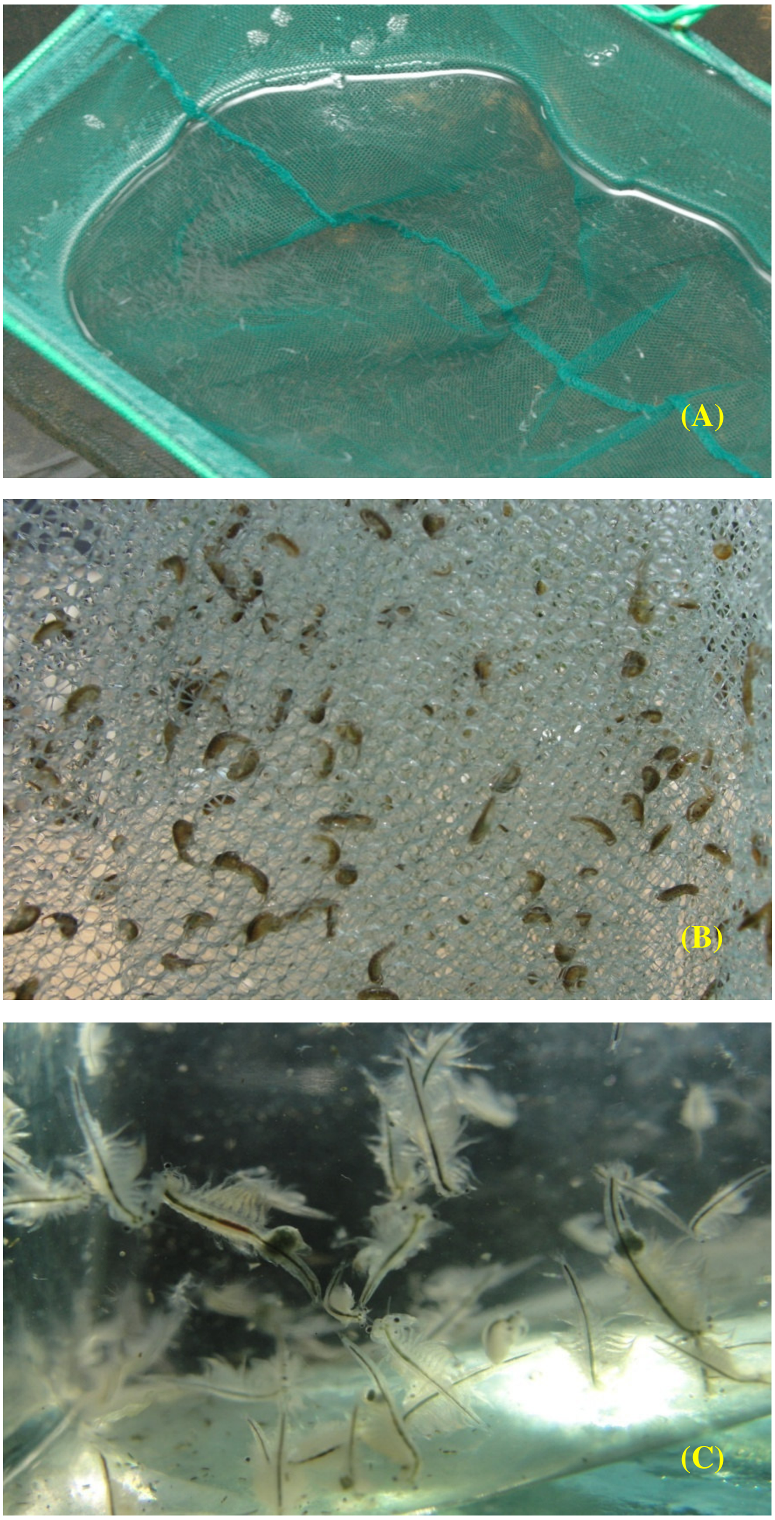

Plate 2.5. Live food organisms used for feeding *S. biaculeatus* in captivity, (A) Mysids, *Mesopodopsis orientalis*, (B) Amphipods, *Grandidierella* sp. and (C) Adult brine shrimps, *Artemia salina*.

N; 73˚48'09.73" E). Mysids were collected with help of hand scoop nets (0.5 mm mesh) and transported to laboratory in aearated plastic containers (100 liters). At the laboratory, collected mysids were maintained in 1500 liter circular FRP tanks and commercial shrimp post larval feed was provided. Collections were made every week to maintain continuous feeding to *S. biaculeatus*.

Amphipods, *Grandidierella* sp.

Live samples were collected from shrimp farm, Nellore District, Andhra Pradesh using a hand scoop net (0.5 mm mesh) and transported to the Aquaculture laboratory, CSIR- National Institute of Oceanography, Goa, in oxygen filled polythene bags. In the laboratory, amphipods were kept in FRP tanks with aeration system for one month to acclimatize for laboratory conditions. Temperature was maintained at 27 ± 1 ºC, salinity 5 ± 1 ppt and pH 7.4-8.6. Salinity of seawater was increased gradually. A commercial shrimp feed was provided daily as food. All collected amphipod individuals were represented by single species and identified as belongs from genus *Grandidierella* (Plate 2.5). *Grandidierella* sp. are known to be tube builders on variety of hard substrates with bottom sediments and therefore make it difficult for harvesting. Therefore, piece of mosquito net clothing (0.5 mm mesh size) was used to raise amphipods in culture system to easy and complete harvesting. Rectangular (1.5m X 1m X 1m) and circular (1.5m X 0.50m) FRP tanks provided with aeration were used. Water quality parameters were temperature 26 ± 1 °C, salinity 16 ± 2 ppt, pH 7.6 ± 0.8, dissolved oxygen 5.7±0.9 mg l^{-1}. FRP Tanks filled with clean, filtered seawater up to 0.30 m tank height. Mosquito net clothing of 30 X 30 cm size were cut and provided as substrate and shelter. Total of 25 cloth piece kept in each tank. Total 300 amphipod individuals (200 females and 100 males) were inoculated in each tank. Commercial shrimp feed (Post Larval feed and starter feed) was provided @ 4% of the body weight. After 35 days of inoculation, density of amphipods in culture tanks increased. Density of amphipods on 30 cm^2 of mosquito net cloth was 103 ± 11 individuals. Harvesting was done simply by washing piece of mosquito net cloth in clean water tub. After complete dislodging from mosquito nets, all the amphipods were passed through a mesh of 1 mm to collect adult individuals only and small juveniles were again released back into culture tanks.

Adult brine shrimps, *Artemia salina*

Artemia salina cyst (Pro 80™, Ocean Star International, Inc. Snowville, USA) were used to obtain *Artemia* nauplii. *Artemia* cysts were hatched in seawater (35 ppt). After 24-36 hours, *Artemia* nauplii were collected with the help of siphon pipe. *Artemia* nauplii were then cultured in outdoor algal culture tank dominated with marine *Chlorella* spp. After 20 days of inoculation, cultured *Artemia* (Plate 2.5) were used for feeding *S. biaculeatus*.

Reproductive behavior of alligator pipefishes

After rearing of alligator pipefishes for one month in laboratory conditions, captive breeding trials were undertaken. For this purpose, alligator pipefishes were kept in all glass tanks of 100 liter capacity with rope mesh (4 mm diameter) as holdfast and continuous aeration was provided. Syngnathid fishes are characterized by monogamous mating pattern (Vincent, 1990; Foster and Vincent, 2004) and similar monogamous mating pattern was observed in case of alligator pipefishes, *S. biaculeatus* (Takahashi *et al*., 2003; Barrows *et al*., 2009). Therefore, male: female ratio was maintained at 1:1 and ten fishes were stocked in each tank. Natural photoperiod was used for adult rearing and breeding trials with live food organisms as ration. Reproductive behaviour in terms of courtship, mating and gestation, fecundity, spawning and inter-mating duration was observed.

Health management

Information on the disease diagnostics playsa significant role in animal health management and disease control. Health related issues were observed during captive rearing of alligator pipefishes. No bacterial or viral diseases were reported during captive rearing, while External Gas Bubble Disease (EGBD) was reported in some adult pipefishes. Two individuals of alligator pipefish, *S. biaculeatus* in the one of the breeding tank got affected with EGBD, one at the ventral side of the head in alligator pipefish (Plate 2.6A) and another at the tip of snout at dorsal side (Plate 2.6B).

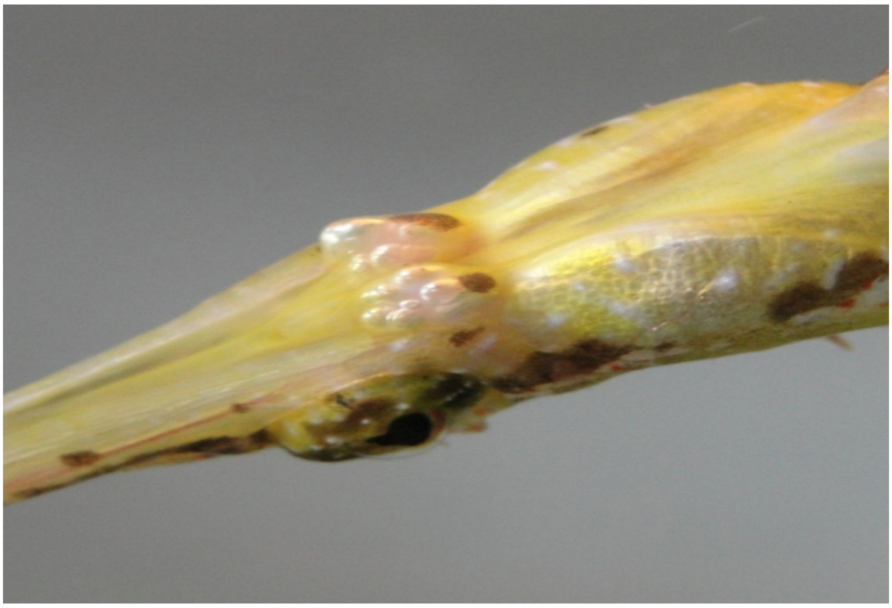

Plate 2.6A. External gas bubble disease observed in *S. biaculeatus* on ventral side of head.

Plate 2.6B. External gas bubble disease observed in *S. biaculeatus* on tip of the snout.

The affected pipefishes were moved to separate tank of 17 liter capacity. Water quality was properly adjusted as that of rearing tank. After acclimatization for 15-20 minutes both fish were released in the hospital tank. 4 mm rope mesh was provided as holdfast. Acetazolamide tablet (trade name Diamox®) which was available at local medical shop was used to treat the animal. One 250 mg Acetazolamide (Diamox®) tablet was used bycrushing into fine powder using mortar and pestle. This fine powder was then mixed properly with some seawater to dissolve and this solution was added in the hospital tank. Then the aeration was adjusted for proper mixing in the tank. After 24 hours, 80 % water was changed and replaced with fresh sea water. Care was taken to avoid difference in water quality. Then fresh solution of Diamox® was added into the tank. This process was repeated for 3-5 days.

Captive rearing of *S. biaculeatus* juveniles

S. biaculeatus juveniles released by broodermale were used in the present study. Mean total length and wet weight were 19.51 ± 0.08 mm and 70.07 ± 0.06 mg, respectively.

Experiment I - Effect of marine zooplankton and *Artemia* nauplii on growth and survival of *S. biaculeatus* juveniles.

Marine zooplankton

Marine zooplankton were collected from Zuari bay using Heron-Tranter net (mesh size, 200 µm) towed horizontally. Composition of different prey organisms is presented in Table 2.11. Collected zooplankton was mostly dominated with copepods (calanoid, cyclopoids and harpactocoid). Zooplankton was further sieved through 400 micron mesh to filter bigger size zooplankton like lucifers, chaetognaths, decapods and jelly fishes.

Table 2.11. Composition (%) of marine zooplankton collected for feeding juveniles of *S. biaculeatus*

Zooplankton taxa	**% composition**
Copepods	66.32
Lucifers	0.64
Cladocerans	19.51
Oikeoplura	1.38
Chaetognaths	3.98
Decapod larvae (Euphausids and shrimps)	1.83
Gastropods	0.16
Brachyuran larvae	0.48
Salps	0.08
Creseis	0.25
Fish larvae	0.15
Bivalve larvae	0.08
Eggs	4.38
Ostracods	0.16
Heteropods	0.16

Artemia nauplii

Artemia cysts were hatched in seawater (35 ppt). After 24-36 hours, *Artemia* nauplii were collected by siphon pipe. Collected *Artemia* nauplii were washed with filtered seawater and then used for feeding pipefish juveniles. Size of *Artemia* nauplii were in the range of 400-475 µm.

Experimental procedure

The *S. biaculeatus* juveniles were active, devoid of yolk sac and started feeding soon after birth. Hence, feeding was done from the first day onwards. Experiment was conducted for rearing juveniles by feeding two different live food organisms *viz*, marine zooplankton and *Artemia* nauplii in all glass tank (capacity 17 liter) containing 10 liter of filtered seawater for 20 days. Experiment was conducted in triplicate for each live food group. *S. biaculeatus* juveniles (total length = 19.51 ± 0.08 mm, weight = 70.07 ± 0.06 mg) were stocked in each experimental tank @ two juveniles per liter. Feeding was done *ad libitum* thrice daily (0800, 1200 and 1800 hours). Density of each live food groups was maintained @ 6-7 nos ml^{-1} in the rearing tank. The prey density was counted in the rearing tanks using a plankton counting chamber viewed through a stereo dissecting microscope (Olympus, Japan). As the density of copepods declined (< 3 nos ml^{-1}), prey was added to the rearing tanks.After siphoning out the waste, 30% of tank water was exchanged daily with fresh seawater before feeding in initial rearing period. Synthetic nylon twine (2 mm diameter) provided were found to be the excellent holdfast during initial rearing. Photoperiod of 16L (0700–2300 hours):8D (2300–0700 hours) was provided to the young ones using a Compact Fluorescent Lamp (CFL) (100W Phillips, India).Adequate aeration was provided using air blowers and optimal water quality parameters were maintained (DO, 5.4 ± 0.5 mg l^{-1}; salinity, 32 ± 1.5 ppt; temperature, 28 ± 02 °C; pH, 7.4 ± 0.8; NO_2–N, 0.02 mg l^{-1} and NH_3/NH_4, 0 mg l^{-1}).After 20 days of rearing period, from each replicate, juveniles were counted, total length was measured using metallic and calibrated foot rule having a least count of 0.5 mm and weight of early fry was measured by using the mono pan balance (Afcoset, The Bombay Burmah Trading Corp. Ltd., India) having accuracy of 0.01 mg and specific growth rate (%) was calculated.

Experiment II - Effect of three different live food organisms on growth and survival of *S. biaculeatus* juveniles.

Experimental procedure

Experiment was conducted to rear 20 days old juveniles by feeding three different live food organisms namely, amphipods, mysid and adult *Artemia* in FRP tank (80 liter capacity) containing 60 liter of filtered seawater for 90 days (Plate 2.7). Experiment was conducted with three replicates for each live food group. Numbers of replicates used in experiment were less due to limitation on collection of parent alligator pipefishes from wild and availability of captive bred juveniles. *S. biaculeatus* juveniles (total length= 64.06 ± 0.50 mm, weight = 144.07 ± 0.06 mg) were stocked in each experimental tank @ one juveniles per three liter (20 juveniles per tank). Feeding was done thrice daily (0800, 1200 and 1800 hours). Density of prey organisms was maintained at 200–300 nos. l^{-1}. After siphoning out the waste, 30 % of fresh seawater was exchanged daily before feeding in initial rearing period. Synthetic nylon twine (4 mm diameter) provided were found to be the excellent holdfast during rearing. Photoperiod of 12L (0700–1900 hours):12D (1900–0700 hours) was provided to the young ones. Adequate aeration was provided using air blowers and optimal water quality parameters were maintained (DO, 5.2 ± 0.7 mg l^{-1}; salinity, 33 ± 1.7 ppt; temperature, 28 ± 02 °C; pH, 7.2 ± 0.5; NO2–N, 0.02 mg l^{-1} and NH3/NH4, 0 mg l^{-1}).After 90 days of rearing period, from each replicate, juveniles were counted. Total length and wet weight was measured by using calibrated foot ruler and mono-pan balance, respectively and specific growth rate (%) was calculated.

Growth parameters

At the end of experimental rearing period, the fish were counted from each replicate and their individual length and weight were recorded. The average value of length and weight were calculated for each replicate of each treatment for analysis of growth

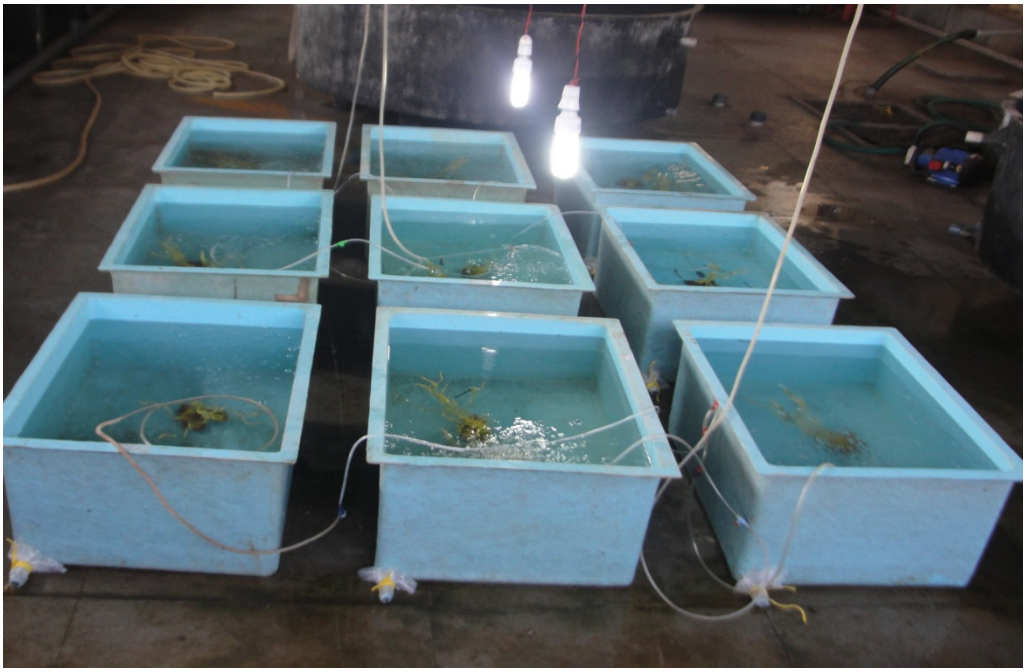

Plate 2.7.Experiemntal tank set up for studying effect of three different live food organisms on growth and survival of *S. biaculeatus* juveniles.

parameters. The growth parameters, such as length gain, weight gain, Specific Growth Rate (SGR) and survival rate were calculated by using the following formulae (Hari and Kurup, 2001). To understand growth profile in terms of total length and weight over period of 110 days (combined for experiment I and II) was separately measured but not included in statistical analysis. Total length and weight was measured at the interval of 20, 30, 50, 70, 90 and 110 days.

Length gain

Total length of juveniles was measured from tip of snout to tip of tail with the help of foot rule having a list count of 0.5 mm.

Length Gain (%) = [(Final length – Initial length) / Initial length] X 100

Weight gain

For recoding wet weight, each juvenile was kept on blotting paper in order to remove excess moisture on body and then immediately put in pre weighed glass beaker having seawater. Increase in weight was recorded as weight of juvenile.

Weight Gain (%) = [(Final weight – Initial weight) / Initial weight] X 100

Specific growth rate (SGR %)

Specific growth rate in terms of % weigh gain day^{-1} was calculated for each treatment. It was derived using following formula.

SGR (%) = [(Log final weight – Log initial weight) / rearing duration in days] X 100

Survival

Survival (%) of fish was calculated using following formula.

Survival rate (%) = Final numbers of juveniles / Initial number of juveniles X 100

Feeding rate

Feeding rate of alligator pipefish juveniles at day 1, 10, 20, 30, 50, 70, 90 and 110 was recorded. Feeding rate was observed on randomly selected five juveniles during daily feeding at 0800, 1200 and 1800 hours for one minute.

Statistical analysis

Mann-Whitney *U* test was used for signifying difference between male and female specimen's length measurements. Morphometric measurements of juveniles were not tested statistically (Mann-Whitney *U* test) against male and females but presented in table. A student *t*-test was applied to determine whether '*b*' values obtained from the linear regression differed significantly from the isometric values (3). The significant difference in regression coefficient 'r^2', intercept '*a*' and regression slope '*b*' between male and female specimens of PB and GoM locations were tested through analysis of covariance (ANCOVA).

Biochemical compositions, total phenolic content and antioxidant activities were presented as mean ± standard deviation. The Pearson correlation analysis was performed between variables and *P*-values lower than 0.05 i.e. ($P<0.05$) were regarded as significant.

The mean weight and length of the alligator pipefish, *S. biaculeatus* juveniles from each replicate of the treatment was calculated. First experiment was conducted as per Students t-test and results of experiment II was analyzed with one way analysis of variance (ANOVA) with Newman-Keuls multiple comparison test, to find out the difference among the mean value of growth and survival of juveniles (Snedecor and Cochran, 1967; Zar, 2004). All statistical calculations were performed using statistical package Statistica 8.0 programme (Stat Soft 8.0) and GraphPad Prism 5.0 statistical software.

Chapter 3
Distribution of alligator pipefish,

Syngnathoides biaculeatus (Bloch, 1785)

along the Indian coast

3.1. Introduction

Pipefishes are known to partition their habitat both within and among seagrass beds according to their morphology, mobility, foraging techniques and prey (Howard and Koehn, 1985; Kendrick and Hydens, 2003). These are poor swimmers and live in narrow habitat range, which enable them to adopt camouflage according to their environment and to maintain stable social structure (Foster and Vincent, 2004). Adult disperse over large distances primarily due to cast adrift by storms or carried away while grasping floating debris, possibly associated with rafts of drifting seaweeds (Kuiter, 2009).

Distribution of syngnathid species is mostly related to availability of their preferred habitat which includes seagrass, mangroves, coral reefs, seaweeds in shallow coastal waters (Foaster and Vincent, 2004; Murugan *et al.*, 2008). The density of these fishes is very low compared to other marine fishes with patchy distribution (Bell and Westoby, 1986; Bell *et al.*, 2003; Foster and Vincent, 2004; Salin and Mohanakumaran, 2006). According to Froese and Pauly (2014) *S. biaculeatus* is a non-migratory species. The relative probabilities of occurrence of *S. biaculeatus* along the west coast of India as per the distribution maps generated (www.fishbase.org) is quite low (0.01 to 0.19 nos. m^2) whereas, along east coast of India (Palk Bay and Gulf of Mannar region) it is high (0.80 to 1.0 nos. m^2).

In India, studies on taxonomy, distribution and resource monitoring of Syngnathid fishes is very limited mostly due to legal restrictions, their small size and camouflage nature, non-commercial value and unawareness. All syngnathid species have been placed under Schedule I of the Indian Wild Life (Protection) Act, 1972. Available information (Sreepada *et al.*, 2002; Salin and Mohanakumaran, 2006; Dhanya *et al.*, 2005; Dhanya, 2008; Murugan *et al.*, 2008) reveal that there have been no systematic work undertaken to ascertain their distribution and diversity along Indian coast. Therefore, in the present study, distribution of *Syngnathoides biaculeatus* along the Indian coast was assessed by direct survey and secondary data resources.

3.2. Results

West coast of India

The survey conducted along the Gujarat coast among the actively engaged fishermen community, revealed no occurrence of the alligator pipefish in the coastal waters of Gujarat. During visits to landing centers and fishing villages in Maharashtra state, it was noticed that there were frequent occurrences of half beak fish, *Hemiramphus* sp. in this region and very often, this species was misidentified as alligator fish, *S. biaculeatus*. Hence, this could be a possible reason for the reporting of this fish along this region.

The survey carried out in Goa suggests an incidental catch of alligator pipefish off Dona Paula, and is the first report of alligator pipefish from the coast of Goa. A detailed profile of the collected specimen in terms of its morphology and meristic count was carried out and is presented in Table 3.1. Further, no specimens of *S. biaculeatus* were observed in by-catch in trawl fishing, gill nets during survey along Karnataka state. The survey conducted along the Kerela coast suggests that this species is known to occur only at Neendakara andSakthikulangara fishing harbours along the south west coast of Kerala. These fishes are very rare in occurrence and found only as one or two specimens in the trawl nets during entire fishing season. It was also reported that these species make an occasional appearance and are not seen in all the seasons along the coast of Kerala and there are no records of its seasonal availability.

New distributional record of the alligator pipefish, *S. biaculeatus* along Goa, central west coast of India

A single male specimen of alligator pipefish (Plate. 3.1) was accidentally caught in the gill net (25 mm mesh size) operated by local fishermen in the Zuari Bay at a depth of 20 m. The collected specimen was observed to be associated with shredded branches of *Sargassum* sp. Reference voucher sample is deposited at the museum, CSIR-National Institute of Oceanography, Goa.

Table 3.1. Comparison of morphometric measurements and meristic counts of *S. biaculeatus* collected from Goa (N = 1) and Palk Bay (N= 30).

Measuring characters	Goa specimen (N=1)	Palk Bay specimens (N=30)
Wet weight (g)	3.71 g	4.22 ±0.47
Morphometric measurements (mm)		
Total length (TL)	179	185±13.35
Maximum body height (H)	6	7±0.46
Maximum body width (iH)	9	12±0.70
Minimum body width (ih)	3.5	5±0.38
Antero-dorsal distance (aD):	63	63±4.92
Post-dorsal distance (pD) :	80	88±6.61
Length of dorsal fin basis (lD)	30	35±1.51
Height of dorsal fin (hD)	4	3±0.39
Length of anal fin basis (lA)	1	1±0.00
Height of anal fin (hA)	2	2±0.00
Length of pectoral fin (lP)	5	6±0.50
Head length (L_H)	36	35±2.19
Occipital height of head (OHH)	7	8±0.73
Head width (Hw)	6	7±0.48
Snout length (SnL)	18	17±1.20
Snout depth (SnD)	3	2±0.44
Mouth width (MW)	2.5	2±0.22
Eye diameter (ED)	6	6±0.48
Post-orbital length (PO):	12	12±0.73
Meristic counts		
Number of rays in dorsal fin (D):	40	39 ± 1.20
Number of rays in anal fin (A):	4	4 ± 0.00
Number of rays in pectoral fin (P)	21	21 ± 1.10
Number of Pre-anal rings (PaR)	16	17 ± 0.00
Number of post-anal rings (PoAR)	35	47 ± 1.17

Plate 3.1. Lateral (A) and dorsal (B) views of *S. biaculeatus* collected from Goa.

Family Syngnathidae Rafinesque, 1810

Body elongate and encased in a series of bony rings; one dorsal fin, usually with 15–60 soft rays; anal fin very small and usually with 2–6 rays; pectoral fin usually with 10–23 rays (the dorsal, anal, and pectoral fins may be absent in adults of some species); no pelvic fins; caudal fin absent in some species; caudal peduncle may be prehensile and employed for holding on to objects when caudal fin is absent; gill openings very small; supra-cleithrum absent; kidney present only on right side, aglomerular. Some species are very colorful. Maximum recorded length about 65 cm (Nelson, 2006).

Syngnathoides biaculeatus **(Bloch, 1785)**

Depressed, tetragonal body; superior and inferior trunk ridges continuous with respective tail ridges; lateral trunk ridge deflected dorsally, ending below superior tail ridge near dorsal fin base; dorsal fin originates on trunk; caudal fin absent; brood below the trunk in front of anal fin in case of male; plates and folds absent; chin with two barbels; variable greenish in color (Schultz, 1943; Dawson, 1985, Murugan *et al.*, 2008).

The present observations on the specimen collected from Goa and its comparison with Palk Bay specimens revealed considerable variations in the morphometric parameters (Table 3.1). Mean body width of specimen collected from Goa (9 mm) was found to be narrower than the specimens collected from Palk Bay (12 ± 0.70 mm). Conspicuous differences in the meristic characters such as body width and number of trunk rings or pre-anal rings of specimens collected from the two localities were discernible (Plate. 3.2). Number of pre-anal or trunk rings in the specimen collected from Goa were 16, while it was 17 in the specimen collected from Palk Bay.

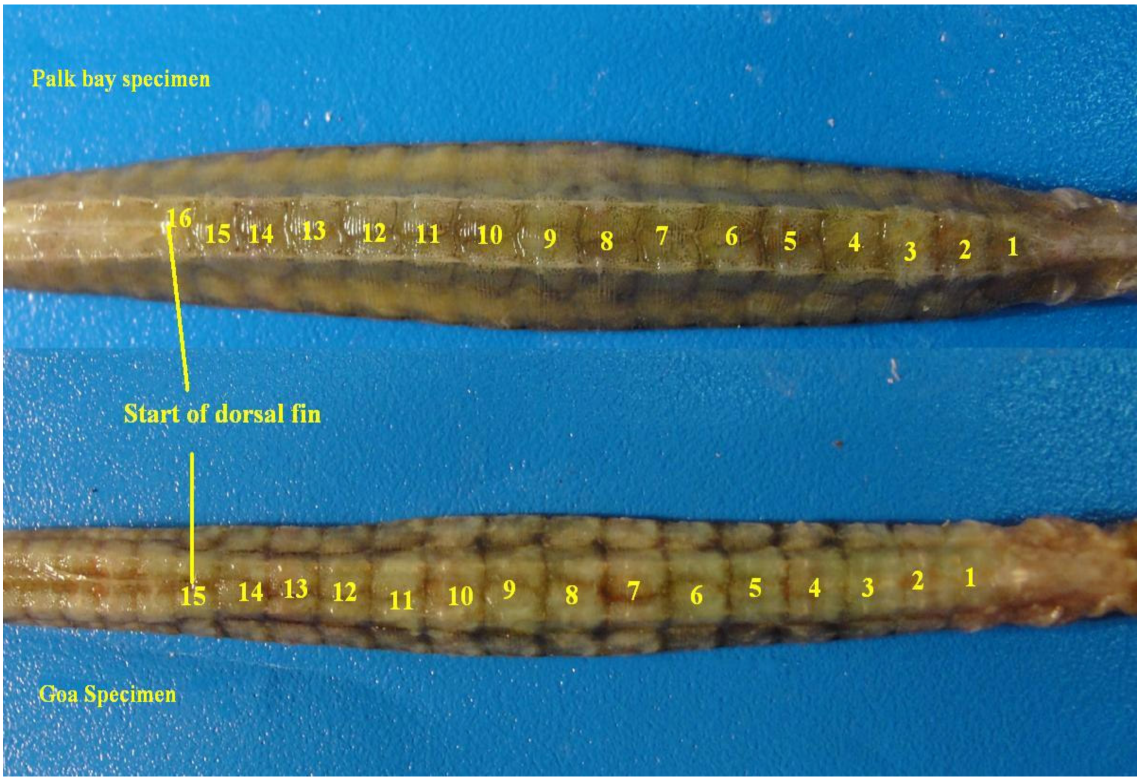

Plate 3.2. Dorsal view of *S. biaculeatus* from Palk Bay (upper) and Goa (lower) indicating the number of pre-anal rings.

East coast of India

One week survey was conducted in coastal area of Pamban, Thondi, Mullimunnai, Parangipettai (Palk Bay region) and Kanyakumari, Tuticorin, Keelakarai and Mandapam and also discussed with local fishermen. Local fishermen know alligator pipefish, *S. biaculeatus* as '*Kadal palli*' in Tamil language; '*Kadal*' means Sea and '*palli*' means pipe. They also told that single *S. biaculeatus*sold clandestinely @ of Rs. 5 to 10 to local dealer. Shredded seagrasses, mostly common eelgrass, *Thalassia hemprichii*, *Cymodocea serrulata* and *Enhalus acoroides* are the major seagrass found along entire coastline, which is the main habitat for *S. biaculeatus*. Dead *S. biaculeatus* were also observed on the coast. *S. biaculeatus* along with seahorses were observed as by-catch from wind driven country trawls locally called as '*Vallams*' (Plate 3.3.). During field visits, apart from *S. biaculeatus,* other syngnathid fishes such as yellow seahorse, *H. kuda*, three spotted seahorse, *H. trimaculatus*, blue spotted pipefish, *Syngnathuscyanospilus*were observed from shrimp trawl by-catch.

No reports of *S. biacuelatus* from Andhra Pradesh and Odisha states were confirmed.

3.3. Discussion

West coast of India

Published report (GEC, 1996) suggest that only three species belong to family Syngnathidae are reported from Gujarat coastal waters namely Yellow seahorse, *Hippocampus kuda*, Blue spotted pipefish, *Syngnathus cyanospilus* and Rough pipefish, *Trachyrhamphus serratus*. Recently, Subburaman *et al*. (2014) reported occurrence of Giraffe seahorse, *H. camelopardalis* for the first time from Gulf of Kachchh, north west coast of India. It is pertinent to note that the above work also did not report occurrence of *S. biaculeatus* in this region.

Available reports (ZSI, 2012) indicate that there are about 1527 species identified from marine waters of Maharashtra, among which, 287 species belongs to Pisces group. Lad and Patil (2013) conducted fish diversity assessment in estuarine area of

Plate 3.3: A) *S. biaculeatus* seen along with seahorses and gastropod shells as by-catch caught in wind driven country trawls. B) Shredded seagrasses, *Thalassia hemprichii*, *Cymodocea serrulata* and *Enhalus acoroides* are the major seagrasses found along the Palk Bay.

Bhayandar and Naigaon of Thane district and reported 53 fish species belonging to 23 families. Naik *et al*. (2002) reported occurrence of yellow seahorse, *H. kuda* in the rocky and marshy shoreline along with seaweed, *Sargassum* sp. from Mirya and Kasarveli creeks in Ratnagiri region of Maharashtra state. Tike *et al*. (2009) studied diversity of minor finfishes and shell fishes along Malvan coast of Maharashtra. They reported a total of 29 species of minor finfishes from Achara, Kolamb, Tarkarli, Devbag and Malvan fishing villages. It is mentioned here that the above reported work does not make a note of occurrence of *S. biaculeatus* along coastal waters of Maharashtra. During direct survey, local fishermen confirm the rare occurrence of seahorses in their nets, but did not confirmreport of *S. biaculeatus* in their area.

The present report of *S. biaculeatus* from Goa is the first record of new distributional range of the *S. biaculeatus* along thecentral west coast of India and is also an extended distributional range from the southwest coast of India. The existing distribution of this species suggests that it is native to Indo-Pacific region (Dawson, 1985; Kuiter, 2009). Published literature from the Indian waters (Dhanya *et al*., 2005; Murugan *et al*., 2008), indicates that *S. biaculeatus* is most common in the Palk Bay and Gulf of Mannar regions along the east coast of India. Bijukumar and Deepthi (2009) recorded this species in the trawl by-catches off Kerala along the south west coast of India albeit in very low numbers. Reviews of published literature (Talwar, 1973; Prabhu and Dhawan, 1974; Ansari *et al*., 1995; Fernandes and Achuthankutty, 2010) on fish biodiversity along the coast of Goa reveal that this species is not known to occur from the coastal waters and wetlands of Goa. Extensive trawl sampling off Goa coast (Ansari *et al*., 1995; Padate, 2010) also suggests the absence of this species along this region. Despite extensive finding efforts (survey along fishing villages, gill net and trawling catch observations) collection of a single specimen reflects that *S. biaculeatus* is very rare in the coastal waters of Goa. At the same time for such a small, camouflagic and un-economic species, confirmation on its new finding is often very difficult to indicate its real expansion or simply that they had been overlooked previously (Tutman *et al*., 2011).

The rare occurrence of *S. biaculeatus* along Goa coast can be linked to the lack of its preferred seagrass habitat. Published literature (Dawson, 1985; Murugan *et al*., 2008) on the habitat preferences of this species suggest that it occurs at shallow depth of 2-5 m in the seagrass bedsof *Thalassia hemprichii*, *Cymodocea serrulata* and *Enhalus acoroides*. Jagtap (1991) reported only two species of seagrasses, *Halophila ovalis* and *H. beccarii* from the mangrove-fringed mudflats along the Mandovi and Terekhol rivers of Goa. Absence of extensive seagrass beds in coastal waters of Goa might have forced *S. biaculeatus* to associate with the seaweeds.

Adults of syngnathid fishes are known to exhibit two different mechanisms for long range dispersal such as drifting by storms and transport while grasping floating debris (Foster and Vincent, 2004). Several other studies (Cho *et al.*, 2001; Ohta and Tachihara, 2004; Wells and Rooker, 2004a, b; Vandendriessche *et al.*, 2007) have reported different syngnathid species associated with floating *Sargassum* and attributed the same for habitat and prey availability. Due to limited mobility, syngnathid fishes are often found attached to seagrasses, seaweeds, corals and any submerged substratum with their prehensile tail (Foster and Vincent, 2004). Long range migration has been earlier reported in some pipefish species (Bayer, 1980; Lazzari and Able, 1990), while some are known to occupy the shallow seagrass beds throughout the year, exhibiting no seasonal movement (Howard and Koehn, 1985). Further, Froese and Pauly (2014) reported that *S. biaculeatus* is a non-migratory species and lack of caudal fin (Kuiter, 1996) is a major obstacle for long range migration. Therefore, the patchy seagrass habitat combined with morphological constraints might hinder the establishment of a sizeable population of *S. biaculeatus* along the Goa coast.

The incidental occurrence of *S. biaculeatus* in coastal waters of Goa could also possibly related to its association with the drifting seaweeds (*Sargassum sp.)* from its known habitats such as Lakshadeep Islands (Jones, 1969) and Kerala (Bijukumar and Deepthi, 2009) along the west coast of India. Hence, in view of the observations made it appears that there is a strong possibility of drifting of *S. biaculeatus* population from these regions. Published reports (Shankar and Shetye, 1999; Shenoi *et al.*, 1999) suggest that, west monsoon current flows around Lakshadeep and then enters into west Indian coastal currents and spread along west coast of India during winter monsoon (January). Alligator pipefish has been previously reported to occur in association with drifting *Sargassum* (Cho *et al.*, 2001; Kuiter, 2009) which could provide possible means of translocation from its known habitats (Lakshadeep Islands and Kerala) to coastal waters of Goa, however need exhaustive survey to further ascertain range extention.

Syngnathid fishes prey upon amphipods, fish larvae and other small invertebrates (Boisseau, 1967; Tipton and Bell, 1988; Do *et al*., 1998; Teixeira and Musick, 2001; present study, Chapter 5). Harmelin-Vivien (1979) recorded zoo-benthos including benthic crustaceans and shrimps as main food items in juveniles and adult alligator pipefish inhabiting Madagascar waters. Ingolfsson and Kristjansson (2002) recorded abundant prey taxa (copepods, crustacean's larvae, ostracods) in and around floating seaweeds. The diversity and abundance of its prey organisms in the coastal and estuarine waters of Goa (Goswami and Padmavati, 1996; Ingole *et al*., 2009) could probably provided adequate food supply to support the occurrence in this region.

The present observation on the specimen collected from Goa and its comparison with Palk Bay specimens revealed considerable variations in the morphometric parameters. These differences could be attributed two factors namely, ontogenic variations in morphological characters and habitat availability. The present specimen from Goa is 177 mm long. The maximum reported size for this species 290 mm TL (Froese and Pauly, 2014). It is apparent from the morphometric characters that the present specimen is a small adult male and its morphological differences with the Palk Bay specimens probably arose due to age differences.

IUCN Red List of Threatened species includes *S. biaculeatus* in 'Data Deficient' category. Hence, its occurrence along Goa, central west coast of India is of bio-geographic significance indicating a possible means of extension of native range, largely attributed to habitat heterogeneity. The rare occurrence of this species in the coastal waters of Goa could be attributed to habitat patchiness and current pattern in Arabian Sea.

According to Karnataka Biodiversity Board (2010) report, there are about 390 fish species belonging to 24 orders and 118 families were identified from Karnataka marine waters. An exhaustive diversified fish assemblage was reported from Netrani Islands, Karnataka, known to possess coral reef patches, also does not support the occurrence of alligator pipefish, *S. biaculeatus* (Zacharia *et al*., 2008; Thomas *et al*., 2011). Further, an assessment of fish diversity survey conducted by Bhat *et al*. (2014) in Aghanashini estuary, Kumta, also did not report any syngnathid fish. On the other hand, Arunachalam *et al*. (1998) reported occurrence of Crocodile tooth pipefish, *Microphis cuncalus* from coastal water of Karnataka state. During present study no specimens of pipefishes was collected due to their very rare status or absence in coastal waters of Karnataka.

Bijukumar and Deepthi (2009) recorded *S. biaculeatus* in the trawl by-catches off Kerala along the south west coast of India albeit in very low numbers. However, finfish diversity assessment in trawl fishery of southern Kerala at four major trawl landing center (Cochin, Munambam, Kalamukku and Neendakara) did not support any sygnathid species (Naomi *et al*., 2011). A Crocodile tooth pipefish, *M. cuncalus* was reported from Ponnani estuary, Kerala, while in the same study area a total of 112 other species of fishes were reported (Bijukumar and Sushama, 2000). Apart from the above mentioned locations, the Fishbase has a record of alligator pipefish, *S. biaculeatus* collected from Kerala coast and deposited at Gulf Coastal Research Laboratory Museum, Mississippi, US. Another specimen has also been deposited at Zoological Survey of India (ZSI, Kolkata) collected from Travancore, Kerala in their museum records. It is evident from the collections made in the present study and above cited literature that the *S. biaculeatus* occurred in Kerala coast; however its distribution is restricted only to southwest coast of the state. Further, the occurrence of seagrass habitat (*Cymodocea* sp and *Syringodium isoetifolium*) in small patches is reported along narrow stretch of south west coast of India (Neendakara and Sakhtikulangara), probably supporting occurrence of *S. biaculeatus* in this region.

East coast of India

During present study, *S. biaculeatus* was reported at all the locations visited during field surveys conducted along the coastal stretch of Tamil Nadu state. This fish is the most common pipefish in Palk Bay and Gulf of Mannar region of Tamil Nadu (Dhanya *et al*., 2005; Murugan *et al*., 2008). Murugan *et al*. (2008) also reported landings of 987, 478 alligator pipefishesyear^{-1} and 5, 45, 295 alligator pipefishes year^{-1}from Palk Bay and Gulf of Mannar, respectively. Thangaraj and Lipton (2010) reported *S. biaculeatus* from Tuticorin coast. According to Murugan *et al*. (2008) *S. biaculeatus* occurred only between Pazhaiyar to Nagapattinam along Coromandal coast. From literature and present survey it is concluded that alligator pipefish, *S. biaculeatus* is most common and abundant pipefish species along Tamil Nadu coast.

Apart from *S. biaculeatus*, few other syngnathid species were reported from coastal waters of Tamil Nadu state. Estuarine pipefish, *I. carce* and crocodile tooth pipefish; *M. cuncalus* was reported from Vellar estuary, Parangipettai, Tamil Nadu (Dhanya *et al.*, 2007). Murugan *et al.* (2008) reported pipefish species in their study, namely, Gray's pipefish, *Halicampus grayi*, blue spotted pipefish, *Hippichthys cyanospilos*= *Syngnathus cyanospilos,* belly barred pipefish, *H. spicifer*, double ended pipefish, *Trachyrhamphus bicoarctatus*, long-nosed pipefish, *T. longirostris*, crested pipefish, *T. serratus*. Habitat suitability including seagrass cover, coral reefs, shallow water and abundant natural food organisms support major syngnathid fish diversity in the Palk Bay and Gulf of Mannar region (Venkataraman and Wafar, 2005; Murugan *et al.*, 2008; Manikandan *et al.*, 2011a, b). Seagrass beds (Palk Bay, Gulf of Mannar, Andaman and Nicobar Island, Lakshadweep Island) form a well suited habitat to alligator pipefish, *S. biaculeatus* where they can camouflage with these long tapes like seagrasses. Published literature on the habitat preferences of this species (Dawson, 1985; Murugan *et al.*, 2008) suggest that it occurs at shallow depth of 2-5 m in seagrass bedsof *Thalassia hemprichii*, *Cymodocea serrulata* and *Enhalus acoroides*. Fourteen species of seagrasses belonging to six genera are reported from Indian marine waters, mostly associated with coral reefs (Jagtap, 1991; Venkataraman and Wafar, 2005; Manikandan *et al.*, 2011a, b). Eleven and thirteen species of sea grasses are reported from Palk bay and Gulf of Mannar respectively. These species includes *Halophila ovalis*, *Halodule pinifolia*, *Syringodium isoetifolium*, *Thalassia hemprichii*, *Cymodocea serrulata*, *Enhalus acoroides* (Jagtap, 1991; Kannan and Thangaradjou, 2006; Manikandan *et al.*, 2011a, b) among which *C. serrulata* was the most dominant species.

According to Andhra Pradesh Biodiversity Board (ABB) there are more than 600 fish species under 300 genera and 121 families reported (ABB, 2009), while no record of *S. biaculeatus* has been made. FishBase database search showed four records of specimens earlier collected from Waltair (Visakhapatnam) during 1976 in GCRL museum, Mississippi, US (FishBase, 2014). This attributed to rare conditions of occurrence of *S. biaculeatus* along Andhra Pradesh coast.

Biodiversity assessment studies conducted by Mohapatra *et al.* (2007) in Chilika Lake, Odisha did not indicate any report of occurrence of alligator pipefish, *S. biaculeatus*. Further, the present observations along the coastal stretch of Odisha state and from the museum records at Chilika development authority, Balugaon and Satpada did not suggest the occurrence of this species from this region.

It is imperative from above survey and available literature on the distribution and occurrence of the pipefishes, the rare nature of occurrence of these species from west coast of India. However, the present observations reveal that this species is well distributed along east coast of India, particularly along Tamil Nadu coast. It is hypothised that the high abundance and occurrence of this species in this region is largly due to habitat complexicity that probably supports population of this species. Further, it appears that, along this region (Palk Bay and Gulf of Mannar), the habitat with patchy occurrence of seagrass beds coupled with favorable ecological conditions sustain high occurrence and distribution of this species.

Chapter 4
Biometric studies and length-weight relationship of alligator pipefish, *Syngnathoides biaculeatus* (Bloch, 1785)

4.1. Introduction

Growth is one of the important parameters that determine the success of a species/population. The variability of this biological parameter has long term implications on the maturity and reproduction of species. Hence, the measurement of the sizes of organisms is a vital part of studies on growth and reproduction, their ecology, behaviour, habitat selection, the effects of tagging or other experimental manipulations, as well as for systematics, population assessments and constructing fisheries models (Lourie, 2003). Among many methods, length-length and length-weight relationships are one of the most common tools used in fishery biology for their greater importance and applicability (Mendes *et al*., 2004).

The length–weight relationship (LWR) is widely used in fisheries research as it facilitates the conversion of growth-in-length equations to growth-in-weight and use in stock assessment models (Pauly, 1993; Petrakis and Stergiou, 1995) as well as estimation of biomass from length observations. LWR is very important in fishery biology and stock assessment of aquatic species as it helps in understanding a wide number of parameters such as estimating growth rates and age structure (LeCren, 1951; Petrakis and Stergiou, 1995).

LWRs of syngnathid fishes generate particular interest because of their unique shape. In case of syngnathid fishes, morphometric and meristic counts have greater significance due to their cryptic nature, camouflaging behaviour and rare occurrence (Lourie et al., 1999). Many species of pipefishes develop filaments on their body, change their color according to habitat (Foster and Vincent, 2004; Kuiter, 2009). These characteristics are basics of their peculiar shape and enable proper identification of the species. The available literature (Cakic *et al*., 2002; Choo and Liew, 2006; Gurkan and Taskavak, 2007; Bijukumar *et al*., 2008; Gurkan, 2008; Ben Amor *et al*. 2011; Vaitheeswaran *et al*., 2012; Vieira *et al*. 2014; Khrystenko *et al*. 2015; Yildiz *et al*. 2015) suggests that there are few observations made on these biometric and LWRs of these fishes, which are very important for comparative growth studies. It is evident that very scanty data is available on length-length relationships (LLR) of syngnathid fishes, andmuch of these studies are with particular reference to seahorses (Froese, 2006; Pawar, 2014), however no such detailed data is available for the pipefish population.

Fulton's condition factor (*K*) is a very useful index for monitoring feeding intensity, age and growth rates in fish and assessing the status of their habitats (Uddin *et al*., 2016). A morphometric study of *S. biaculeatus* from the coastal waters of Papua New Guinea (Barrows *et al*., 2009) was restricted to the determination of '*b*' value from a small population size (N = 41). Dhanya (2008) provided information on LWR in *S. biaculeatus* from Palk Bay, southeast coast of India. Although the LWR, '*b'* value, LLR and *K* reflect the growth conditions within a particular population on a wider spectrum, a combined analysis of these indices could provide better insight on the status of growth parameters in such populations. The present study attempts a comprehensive analysis of biological parameters (LLRs, LWRs and *K*) in *S. biaculeatus* populations and would provide baseline information on these issues.

4.2. Results

Morphometric measurements

Minimum, maximum and mean values of morphometric measurements of male, female and juvenile *S. biaculeatus* specimens, collected from Palk Bay, Gulf of Mannar, Kerala and Goa are presented in Tables 4.1, 4.2, 4.3, and 4.4, respectively. There were no significant differences in the morphometric measurment and meristic counts between the Palk Bay and Gulf of Mannar specimens. However, the differences in morphometric measurments between the sexes (male and female) were found to be significant at all locations. Post-dorsal lengths (pD) in male specimens (97 ± 12.09 mm) are considerably greater than female specimens (87.26 ± 8.10 mm) from Palk Bay. Similarly, pD in male specimens (96.63 ± 12.02 mm) from the Gulf of Mannar region are greater than female specimens (88.23 ± 6.81 mm).

Table 4.1. Morphometric measurements of *S. biaculeatus* from Palk Bay, Tamil Nadu (N= 120).

Morphometric Measurements	Males (N =41)				Females (N=57)				Juveniles (N =22)			
	Min.	Max.	Mean ± SD	Mean as % of TL	Min.	Max.	Mean ± SD	Mean as % of TL	Min.	Max.	Mean ± SD	Mean as % of TL
Wet weight (g)	3.99	6.88	5.64±0.85	-	3.79	6.72	5.30±0.77	-	1.58	2.18	2.21±0.37	-
TL(mm)	160	250	205±25.78	-	159	230	184±17.15	-	90	145	122±13.60	-
H (mm)	6	10	8±0.97	3.86	6.37	9.15	7.38±0.67	4.01	2.81	5.39	4.27±0.72	3.49
iH (mm)	10	17	13±1.65	6.49	10.11	15.69	12.26±1.22	6.66	4.28	8.75	6.75±1.31	5.52
ih (mm)	4	7	5±0.71	2.67	4.11	6.53	4.95±0.51	2.69	2.39	3.84	3.23±0.36	2.64
aD (mm)	55	85	70±9.14	34.26	53.57	78.78	63.35±5.94	34.43	30.18	48.62	41.68±4.82	34.09
pD (mm)	75	122	97±12.09	47.21	74.44	109.78	87.26±8.10	47.42	36.68	65.28	53.51±7.59	43.77
lD (mm)	21	34	27±3.61	13.37	21.82	31.79	25.41±2.36	13.81	12.71	20.47	16.55±1.84	13.54
hD (mm)	3	4	3±0.49	1.67	2.27	3.82	2.81±0.35	1.53	1.58	2.69	2.25±0.29	1.84
lA (mm)	1	1	1±0.16	0.52	0.91	1.31	1.05±0.10	0.57	0.53	0.86	0.65±0.09	3.94
hA (mm)	1	3	2±0.33	1.04	1.79	2.62	2.09±0.19	1.14	1.06	1.71	1.30±0.18	1.07
lP (mm)	5	10	7±1.02	3.42	5.01	7.61	6.19±0.60	3.36	3.18	5.12	4.07±0.47	3.32
L_H (mm)	30	47	38±4.79	18.66	29.42	43.19	34.77±3.26	18.90	17.47	28.14	23.02±2.52	18.83
OHH (mm)	6	10	8±1.05	4.00	6.39	10.47	7.71±0.89	4.19	3.71	5.97	5.02±0.56	4.10
Hw (mm)	5	9	7±1.06	3.64	5.98	9.15	7.02±0.69	3.82	3.44	5.54	4.69±0.53	3.84
SnL (mm)	15	24	19±2.34	9.27	14.54	21.63	17.46±1.74	9.49	8.47	13.64	11.18±1.22	9.14
SnD (mm)	2	4	3±0.42	1.22	1.79	3.73	2.48±0.47	1.35	1.03	2.02	1.58±0.28	1.29
MW (mm)	1	3	2±0.35	1.03	1.39	2.62	1.91±0.30	1.04	1.01	1.64	1.25±0.17	1.02
ED (mm)	5	8	6±0.86	3.14	4.60	7.84	5.86±0.71	3.18	2.65	4.34	3.70±0.44	3.02
PO (mm)	10	16	13±1.63	6.24	10.00	14.61	11.74±1.08	6.38	6.35	10.24	8.09±0.96	6.62
ID (mm)	4	6	5±0.59	2.27	3.63	5.22	4.22±0.38	2.29	2.12	3.41	2.75±0.31	2.25

Table 4.2. Morphometric measurements of *S. biaculeatus* from Gulf of Mannar, Tamil Nadu (N= 97).

Morphometric Measurements	**Males (N =37)**				**Females (N =44)**				**Juveniles (N =16)**			
	Min.	Max.	Mean ± SD	Mean as % of TL	Min.	Max.	Mean ± SD	Mean as % of TL	Min.	Max.	Mean ± SD	Mean as % of TL
Wet weight (g)	4.57	6.87	5.83±0.75	-	3.67	6.32	5.40±0.64	-	1.48	2.78	2.10±0.44	-
TL(mm)	165	250	204±25.61	-	159	220	185.82±13.94	-	90	140	118.44±17.50	-
H (mm)	5.99	10.06	8.01±1.08	3.93	6.19	9.55	7.63±0.73	4.10	2.81	5.00	4.24±0.65	3.59
iH (mm)	10.26	16.68	13.36±1.77	6.55	10.61	14.81	12.32±0.97	6.62	4.28	8.75	7.29±1.31	6.18
ih (mm)	4.27	6.95	5.47±0.71	2.68	3.98	6.29	5.09±0.51	2.74	2.39	3.75	3.20±0.43	2.71
aD (mm)	56.23	85.43	70.73±8.54	34.67	53.31	74.16	62.89±4.55	33.81	30.18	46.26	39.71±5.15	33.66
pD (mm)	76.08	119.25	96.63±12.02	47.37	75.14	105.60	88.23±6.81	47.44	36.68	68.12	57.11±9.41	48.40
lD (mm)	20.53	33.39	27.30±3.88	13.38	21.22	31.44	25.81±2.08	13.88	12.30	18.13	15.65±1.92	13.26
hD (mm)	2.50	4.33	3.33±0.52	1.63	2.31	4.17	3.11±0.48	1.67	1.58	2.51	2.15±0.29	1.82
lA (mm)	0.86	1.44	1.10±0.16	0.54	0.88	1.25	1.05±0.08	0.57	0.43	0.65	0.56±0.06	0.47
hA (mm)	1.72	2.87	2.21±0.31	1.09	1.76	2.51	2.09±0.15	1.13	0.85	1.30	1.11±0.13	0.94
lP (mm)	5.13	9.74	6.99±1.12	3.42	5.06	8.36	6.51±0.74	3.50	2.97	4.38	3.80±0.45	3.22
L_H (mm)	30.77	47.42	38.44±4.88	18.84	30.05	43.00	35.33±2.91	19.00	17.39	25.62	22.04±2.77	18.68
OHH (mm)	6.42	10.44	8.26±1.20	4.05	6.19	9.91	7.82±0.82	4.20	3.71	5.63	4.83±0.62	4.09
Hw (mm)	5.99	9.34	7.57±0.98	3.71	5.95	8.28	6.93±0.56	3.73	2.95	4.34	3.85±0.44	3.26
SnL (mm)	15.39	23.66	19.04±2.37	9.33	14.59	21.50	17.70±1.48	9.52	8.47	13.13	11.20±1.52	9.49
SnD (mm)	1.68	3.24	2.34±0.43	1.15	1.84	3.59	2.55±0.52	1.37	1.03	1.88	1.59±0.25	1.34
MW (mm)	1.42	2.87	2.14±0.39	1.05	1.38	2.32	1.87±0.26	1.00	0.85	1.25	1.10±0.13	0.93
ED (mm)	5.12	8.34	6.48±0.89	3.18	4.60	7.16	5.89±0.61	3.17	2.65	4.06	3.49±0.45	2.96
PO (mm)	10.15	15.81	12.82±1.72	6.28	10.11	14.34	11.90±0.95	6.40	5.73	8.44	7.36±0.85	6.24
ID (mm)	3.42	5.75	4.50±0.67	2.20	3.54	5.38	4.34±0.40	2.33	2.12	3.19	2.73±0.36	2.31

Table 4.3. Morphometric measurements of *S. biaculeatus* collected from Kerala.

Measurements	*S. biaculeatus*(N= 18)			
	Min.	Max.	Mean ± SD	Mean as % of TL
Wet weight (g)	3.76	6.49	5.28 ±0.64	-
Morphometric measurements (mm)				
TL	159.00	230.00	187.11±18.54	-
H	5.96	8.65	7.44±0.77	3.98
iH	10.43	14.38	12.20±1.13	6.52
ih	4.13	6.16	5.05±0.54	2.70
aD	53.31	75.99	63.77±6.52	34.10
pD	75.37	111.92	89.12±9.53	47.66
lD	21.63	29.79	25.32±2.44	13.54
hD	2.31	4.12	3.07±0.44	1.64
lA	0.75	1.13	1.01±0.09	0.54
hA	1.49	2.31	2.04±0.19	1.09
lP	5.06	7.58	6.40±0.69	3.42
L_H	30.34	42.09	35.11±3.42	18.78
OHH	6.45	9.74	7.69±0.95	4.11
Hw	5.23	7.68	6.82±0.66	3.65
SnL	15.17	21.57	17.46±1.72	9.34
SnD	1.84	3.17	2.43±0.44	1.30
MW	1.38	2.26	1.91±0.26	1.02
ED	4.60	6.67	5.76±0.64	3.08
PO	10.07	13.87	11.72±1.16	6.27
ID	3.67	5.24	4.21±0.41	2.25

Table 4.4. Morphometric measurements of *S. biaculeatus* collected from Goa.

Measurements	*S. biaculeatus* (N =1)
Wet weight (g)	3.71
Morphometrics measurements (mm)	
TL	179
H	6
iH	9
ih	3.5
aD	63
pD	80
lD	30
hD	4
lA	1
hA	2
lP	5
L_H	36
OHH	7
Hw	6
SnL	18
SnD	3
MW	2.5
ED	6
PO	12
ID	3.2

Meristic counts

The meristic counts of *S. biaculeatus* collected from Palk Bay, Gulf of Mannar, Kerala and Goa are presented in Tables 4.5, 4.6, 4.7 and 4.8, respectively. The mean numbers of pre-anal rings (PaR) in males, females as well as juveniles collected from all locations are 16, thus indicating lack of variations in these counts irrespective of life stages. On the other hand, mean number of post-anal rings (PoAR) are varied between males/female (47) and juveniles (39) for the sample collected from Palk Bay and Gulf of Mannar region. However, the numbers of PoAR collected from the Kerala coast (pooled samples) were slightly lesser (46). Similarly, median values of pectoral and dorsal fin ray counts in males exceeded those of females by one. Results of Mann Whitney *U* test revealed no significant differences ($P > 0.05$) in the counts of PaR, PoAR, pectoral fin rays and dorsal fin rays between different populations.

Length-length relationship

Positive correlations between all LLRs were observed for all specimens studied from Palk Bay (Table 4.9), Gulf of Mannar (Table 4.10) and Kerala (Table 4.11) coasts.

Length-Weight relationship and Fulton's condition factor

The estimated regressions for both male and female LWRs were significant ($r^2 < 0.95$). The LWRs of all the *S. biaculeatus* specimens (N = 235) collected from Palk Bay, Gulf of Mannar and Kerala are described by the following equation, $W (g) = -1.51 \times TL^{1.75} (cm)$. The student *t*-test revealed negative allometric growth in both male and female population. There were no significant differences between the '*b*' values of male and female specimens observed. ANCOVA test revealed no difference in regression coefficient, intercept and regression slope value of male and female specimens from PB and GoM ($P > 0.05$). The estimated values of LWRs are presented in Table 4.12. Overall, '*b*' value of specimens collected from Palk bay, Gulf of Mannar, and Kerala is estimated to be 1.75.

Table 4.5. Meristics counts of *S. biaculeatus* collected from Palk Bay, Tamil Nadu (N= 120).

Meristic parameters	**Meristic count, males (N =41)**				**Meristic count,females (N =57)**				**Mann-Whitney *U* test**	**Meristic count,juveniles (N =22)**			
	Min.	Max.	Mean ± SD	Median	Min.	Max.	Mean ± SD	Median		Min.	Max.	Mean ± SD	Median
Dorsal fin rays (D)	38	42	40±2	39	38	41	39±1	39	*P*> 0.05	37	40	39±1	39
Anal fin rays (A)	4	4	4	4	4	4	4.00	4	*P*> 0.05	4	4	4	4
Pectoral fin rays (P)	19	22	20	20	19	22	19±1	20	*P*> 0.05	18	20	19	19
Pre-anal rings (PaR)	16	17	16	16	16	16	16	16	*P*> 0.05	16	16	16	16
Post-anal rings (PoAR)	44	50	47±3	47	43	49	47±2	48	*P*> 0.05	37	42	39±1	39

Table 4.6. Meristics counts of *S. biaculeatus* collected from Gulf of Mannar, Tamil Nadu (N= 97)

Meristic parametser	**Meristic count,males (N=37)**				**Meristic count,females (N =44)**				**Mann-Whitney *U* test**	**Meristic count,juveniles (N =16)**			
	Min.	Max.	Mean ± SD	Median	Min.	Max.	Mean ± SD	Median		Min.	Max.	Mean ± SD	Median
Dorsal fin rays (D)	38	41	39±1	39	38	40	39±1	39	*P* .> 0.05	38	40	39±1	39
Anal fin rays (A)	4	4	4	4	4	4	4	4	*P*> 0.05	4	4	4	4
Pectoral fin rays (P)	19	21	20±1	19	19	21	20±1	19	*P* > 0.05	19	20	19	19
Pre-anal rings (PaR)	16	17	16	16	16	17	16	16	*P*> 0.05	16	16	16	16
Post-anal rings (PoAR)	45	49	47±2	46	44	49	47±1	47	*P*> 0.05	37	42	39±1	39

Table 4.7. Meristics counts of *S. biaculeatus* (N= 18) collected from Kerala

Meristic parameters	**Meristic count**			
	Min.	Max.	Mean ± SD	Median
Dorsal fin rays (D)	38	41	39±1	39
Anal fin rays (A)	4	4	4	4
Pectoral fin rays (P)	19	22	20±1	20
Pre-anal rings (PaR)	16	17	16	16
Post-anal rings (PoAR)	44	48	46±1	46

Table 4.8. Meristics counts of *S. biaculeatus* (N = 1) collected from Goa.

Meristics parameters	**Meristic count**
Dorsal fin rays (D)	40
Anal fin rays (A)	4
Pectoral fin rays (P)	21
Pre-anal rings (PaR)	16
Post-anal rings (PoAR)	35

Table 4.9. Length-length relationships (LLR) of *S. biaculeatus* collected from Palk Bay, Tamil Nadu.

Morphometric relationships	**Male (N = 41)**		**Female (N = 57)**		**Juveniles (N = 22)**		**Pooled (N = 120)**	
	r	**Regression equation**	**r**	**Regression equation**	**r**	**Regression equation**	**r**	**Regression equation**
aD on TL	0.95	aD = -0.72 +0.34 TL	0.94	aD = 1.12 + 0.33 TL	0.98	aD = -1.18 + 0.35 TL	0.98	aD = -0.36 + 0.34 TL
pD on TL	0.97	pD = 2.05 +0.46 TL	0.98	pD = 0.67 + 0.46 TL	0.77	pD = -6.41 + 0.49 TL	0.98	pD = -6.95 + 0.50 TL
SnL on L_H	0.97	SnL = 0.61 + 0.48 L_H	0.87	SnL = 0.16 + 0.49 L_H	0.99	SnL =0.01 + 0.48 L_H	0.97	SnL = -0.32 + 0.50 L_H
OH on L_H	0.89	OH = 0.27 + 0.20 L_H	0.76	OH = 0.52 + 0.20 L_H	0.93	OH =0.11 + 0.21 L_H	0.86	OH = 0.24 + 0.21 L_H
PO on L_H	0.94	PO = 0.10 + 0.33 L_H	0.97	PO = 0.38 +0.32 L_H	0.99	PO = -0.11 + 2.02 L_H	0.98	PO = 0.73 + 0.31 L_H
ED on L_H	0.92	ED = -0.19 +0 .17 L_H	0.69	ED = -0.48 + 0.18 L_H	0.76	ED =-0.17 + 0.15 L_H	0.93	ED = -0.39 + 0.17 L_H
iH on aD	0.91	iH = 1.12 + 0.17 aD	0.80	iH = 0.53 + 0.18 aD	0.64	iH = -2.34 + 0.21 aD	0.93	iH = -1.75 + 0.21 aD
ID on aD	0.97	ID = 0.08 + 0.38 aD	0.86	ID = 2.08 + 0.36 aD	0.84	ID = 2.80 + 0.32 aD	0.96	ID = 0.85 + 0.38 aD
ED on OH	0.74	ED = 0.60 + 0.71 OH	0.77	ED = 0.62 + 0.41 OH	0.93	ED = -0.16 + 0.76 OH	0.79	ED = 0.17 + 0.74 OH

Table 4.10. Length-length relationships (LLR) of *S. biaculeatus* collected from Gulf of Mannar, Tamil Nadu.

Morphometric relationships	**Males (N = 37)**		**Females (N = 44)**		**Juveniles (N = 16)**		**Pooled (N = 97)**	
	r	**Regression equation**	**r**	**Regression equation**	**r**	**Regression equation**	**r**	**Regression equation**
aD on TL	0.96	aD = 3.72 +0.32 TL	0.88	aD = 5.84 + 0.30 TL	0.87	aD = 7.14 + 0.27 TL	0.97	aD = -0.76 + 0.34 TL
pD on TL	0.97	pD = 1.99 +0.46 TL	0.98	pD = –1.90 + 0.48 TL	0.86	pD = –2.13 + 0.50 TL	0.98	pD = 1.98 + 0.46 TL
SnL on L_H	0.98	SnL = 0.55 + 0.48 L_H	0.89	SnL = 0.62 + 0.48 L_H	0.98	SnL = –0.89 + 0.54 L_H	0.98	SnL = 0.58 + 0.48 L_H
OH on L_H	0.74	OH = 0.12 + 0.21 L_H	0.66	OH = –0.37 + 0.23 L_H	0.99	OH = –0.11 + 0.22 L_H	0.89	OH = 0.24 + 0.21 L_H
PO on L_H	0.90	PO = –0.03 + 0.33 L_H	0.97	PO = 0.43 +0.32 L_H	0.93	PO = –0.78 + 0.29 L_H	0.97	PO = 0.08 + 0.33 L_H
ED on L_H	0.90	ED = –0.23 +0 .17 L_H	0.68	ED = –0.24 + 0.17 L_H	0.97	ED =–0.04 + 0.16 L_H	0.94	ED = -0.43 + 0.17 L_H
iH on aD	0.90	iH = –0.57 + 0.19 aD	0.87	iH = 2.09 + 0.16 aD	0.86	iH = –2.12 + 0.23 aD	0.93	iH = -0.28 + 0.19 aD
ID on aD	0.94	ID = –3.80 + 0.43 aD	0.77	ID = 0.47 + 0.40 aD	0.96	ID = 1.11 + 0.33 aD	0.94	ID = 0.36 + 0.39 aD
ED on OH	0.76	ED = 1.43 + 0.61 OH	0.75	ED = 1.95 + 0.50 OH	0.87	ED = 0.33+ 0.06 OH	0.80	ED = 0.11 + 0.74 OH

Table 4.11. Length-length relationships (LLR) of *S. biaculeatus* collected from Kerala.

Morphometric relationships	**Pooled (N = 18)**	
	r	**Regression equation**
aD on TL	0.95	aD = –0.45 + 0.34TL
pD on TL	0.97	pD = –5.81 + 0.50TL
SnL on L_H	0.95	SnL = 0.19 + 0.49 L_H
OH on L_H	0.59	OH = 0.17 + 0.21 L_H
PO on L_H	0.85	PO = 0.66 + 0.31 L_H
ED on L_H	0.79	ED = –0.07 + 0.16 L_H
iH on aD	0.84	iH = 2.00 + 0.15 aD
ID on aD	0.84	ID = 3.44 + 0.34 aD
ED on OH	0.87	ED = 2.58 + 0.41 OH

Table 4.12. Length- weight relationships (LWR) of *S. biaculeatus* (N = 235) for Palk Bay, Gulf of Mannar and pooled for Indian coast.

Location	Sex	Nos.	Total Length (cm)		Weight (g)		a	*b*	*b*range	r^2	Fulton's Condition factor 'K'
			Min	Max	Min	Max					
Palk bay	Males	41	16	25	3.99	6.91	1.03	1.17	1.08 – 1.26	0.94	0.69
	Females	57	15.9	23.0	3.79	6.72	0.17	1.56	1.42 – 1.71	0.89	1.08
	Juveniles	22	9	14.5	1.58	2.81	–2.59	1.40	1.20 – 1.60	0.91	1.35
	Pooled	120	9	25	1.58	6.91	–1.52	1.75	1.67 – 1.83	0.93	0.85
Gulf of Mannar	Males	37	16.5	25	4.57	6.87	–0.73	1.14	1.02 – 1.26	0.91	0.68
	Females	44	15.9	22	3.67	6.28	–1.31	1.61	1.42 – 1.80	0.87	0.84
	Juveniles	16	9	14	1.48	2.78	–1.08	1.31	1.07 – 1.54	0.91	1.27
	Pooled	97	9	25	1.48	6.87	–1.52	1.75	1.66 – 1.84	0.94	0.84
Pooled from all localities		235	9	25	1.48	6.91	–1.51	1.75	1.69 – 1.80	0.93	0.84

Fulton's condition factor ranged from 0.65 to 1.35 (0.85 for pooled samples) for Palk Bay specimens, and from 0.68 to 1.27 (0.84 for pooled samples) for Gulf of Mannar specimens (Table 4.12).

4.3. Discussion

An appropriate understanding of species conditions, biometric characters and LWR, enable species identification, to design appropriate guidelines to modify fishing practices, identify critical habitats to designate protective marine reserves and facilitate the assessment of captive breeding potential of ecologically important population. There are a few studies related to biometric and LWR of syngnathid fishes (Cakic *et al.*, 2002; Choo and Liew, 2006; Gurkan and Taskavak, 2007; Bijukumar *et al.*, 2008; Gurkan, 2008; Ben Amor *et al.*, 2011; Vieira *et al.*, 2014; Khrystenko *et al.*, 2015; Yildiz *et al.*, 2015) those emphasis the need of such studies for better conservation measures.However, biometric, LLR and LWR studies in *S. biaculeatus* are lacking. In the present study, morphometric relationships were established for *S. biaculeatus* collected from different locations of coastal India, considering the lack of information on these aspects. Although, the present day *Syngnathoides* is a single genus in the family Syngnathidae, establishment of morphometric characters, LLR and LWR relations may facilitate the identification of another congeneric species and enable comparison with conspecific individuals from different parts of the world.

Taxonomic and population studies related to Indian syngnathid fishes are limited, and most of these focus on the seahorses (Bijukumar *et al.*, 2008; Vaitheeswaran*et al.*, 2012). On the other hand, published literature (Jones, 1969; Murugan *et al.*, 2008) suggested about 13 species of pipefishes have been reported from Indian marine waters. However, with the exception of Dhanya (2008), there are no other comprehensive studies related to morphometric and meristic characters of pipefishes in India. The observations made in the present study revealed significant differences ($P < 0.05$) in mean total lengths (TL) of male and female specimens of *S. biaculeatus* collected from both locations namely, Palk Bay and Gulf of Mannar region, suggesting that the males grow bigger in size compared to females irrespective of their region of occurrence.

Among meristic counts, PoAR counts of male and female *S. biaculeatus* specimens from Palk Bay and Gulf of Mannar region were found to be different. This variation in PoAR count is probably due to a difference in post-dorsal lengths of the male and female specimens, indicating the increased size of the tail region. Numbers of post-anal rings (PoAR) are lesser in juvenile specimens due to their smaller size, indicating that growth in syngnathid fishes is associated with increase in body and tail rings (Choo and Liew, 2006). Gurkan (2008) studied morphometric and meristic characters of three pipefish species namely Greater pipefish, *Syngnathus acus*, Broadnosed pipefish, *S. typhle* and Straightnosed pipefish, *Nerophis ophidion* from the Aegean Sea, Turkey and reported variations in the number of pre-anal (PaR) and post-anal (PoAR) rings of male and females of *S. ancus*. Cakic *et al*. (2002) studied the biometric characters (18 morphometric and 7 meristic counts) of the Black-striped pipefish, *Syngnathus abaster* populations from Black Sea, Danube River and Azove Sea, Ukraine and reported significant differences in three out of seven meristic counts and in 16 out of 18 morphometric measurements. In the present study, significant differences ($P< 0.05$) have been observed between morphometric characters of males and females from the same locality, however, there are no observed significant differences in meristic counts.

It is necessary to use standard measures for all populations to render the results more reliable when making comparisons between populations (Kara and Bayhan, 2008). Therefore, the Length-Length Relationship (LLR) of species under various environmental conditions should be known. The LLR is also of great importance for comparative growth studies (Moutopoulos and Stergiou, 2002). Information on LLRs of syngnathid fishes is scanty (Pawar, 2014), however; no reports are available on LLRs of pipefishes. Therefore LLRs of *S. biaculeatus* established during the present study could facilitate to provide better insight regarding growth patterns and for assessment of different *S. biaculeatus* populations around the world.

During the present study, a total of 235 alligator pipefishes from Palk Bay (120 specimens, 41 males, 57 females and 22 juveniles), Gulf of Mannar (97 specimens, 37 males, 44 females and 16 juveniles) and Kerala (18 specimens) were used for establishing LWR. The observations made in the present study revealed that the populations of *S. biaculeatus* showed negative allometric growth pattern (b = 1.75 for N= 235). In contrast, Dhanya (2008) reported relatively high 'b' values (males - 3.174;

females - 3.001 and juveniles - 2.483) based on a LWR study of 981 specimens of *S. biaculeatus* (400 males, 347 females and 234 juveniles) from Palk Bay. Similarly, Barrows *et al.* (2009) reported a very high '*b*' value of 4.074 for *S. biaculeatus* (N= 41; 18 males, 21 females and 2 juveniles) collected from Bootless Bay, Papua New Guinea. Plausible explanations for such high '*b*' values reported by these authors were mainly due to the inclusion of pregnant males in the LWR analysis and favourable environmental factors at sampled locations. Details of previously attempted LWR studies of syngnathid fishes are presented in Table 4.13.

In case of other pipefish species, Gurkan and Taskavak (2007) reported '*b*' values of 2.42, 3.00 and 3.54 for *Nerophis ophidian*, *Syngnathus typhle* and *S. acus*, respectively from Aegean Sea, Turkey. Ben Amor *et al.* (2011) reported '*b*' values of 2.62, 2.64, 1.836 and 5.476, for *Syngnathus abaster*, *S. acus*, *Syngnathus typhle* and *N. ophidion,* respectively from Tunisian waters. Khrystenko *et al.* (2015) reported positive allometric growth pattern (*b*> 3 3.017 to 3.338) in LWR of *S. abaster* populations in Dnieper river basin, Ukraine. In another study, Yildiz *et al.* (2015) reported '*b'* value of 3.41 for *S. acus* from Western Black Sea, Turkey. LWRs of four pipefish species from Ria Formosa, SW Iberian coast, Portugal has been studied by Vieira *et al.* (2014). They reported '*b'* values of 3.11, 3.36, 3.34 and 3.35 for *N. ophidian*, *S. abaster*, *S. acus* and *S. typhle,* respectively. From the above studies, it can be deduced that values of '*b'* in LWR vary greatly among conspecific populations from different geographical locations (Table 4.13). Several authors (Tesch, 1971; Wootton, 1998; Froese, 2006; Dhanya, 2008; Karachle and Stergiou, 2012; Vieira *et al*., 2014; Khrystenko *et al*., 2015; Yildiz *et al*., 2015) opined that the LWR depends upon environmental factors (temperature, salinity) and biological processes (season, food availability, habitats, gonad development, health) as well as differences in the sizes of specimens subjected to LWR analysis. In present study negative allometric growth pattern was observed in case of *S. biaculeatus*, this probably can be attributed to the species morphology and the nature of habitat.Increased degradation of seagrass habitat of due to bottom shrimp trawling, wind driven country trawls in the Palk Bay and Gulf of Mannar regions (D'Souza *et al*.,

Table 4.13.Length-weight relationships (LWR) in pipefishspecies.

Species	Numbers	Locality	Length range cm (mean)	Weight range g (mean)	'*b*' value	Growth pattern	References
Syngnathoides biaculeatus	**235**	**Palk Bay, Gulf of Mannar and Kerala, India**	**9–25 (18.18)**	**1.48 – 6.91 (5.02)**	**1.75**	**Negative allometric**	**Present study**
S. biaculeatus,Male	347	Palk Bay, India	13.2–24.8 (18.74)	–	3.17	Positive allometric	Dhanya (2008)
Female	400		13–22.8 (17.83)		3.00	Isometric	
Juveniles	234		1.7–15.5 (6.58)		2.48	Negative allometric	
S. biaculeatus	41	Bootless Bay, Papua New Guinea	–	–	4.07	Positive allometric	Barrows *et al*., (2009)
Syngnathus acus	570	Bay of Izmir, Aegean coast, Turkey	3.3–25.6 (10.1)	0.01–12.29 (0.60)	3.54	Positive allometric	Gurukan and Taskavak, (2007)
S. typhle	125		4–25.8 (15.53)	0.01–8.2 (1.49)	3.00	Isometric	
Nerophis ophidion	86		7.8–21.4 (14.57)	0.06–0.83 (0.35)	2.42	Negative allometric	
S. abaster	47	River Danube, Ukrain	6.08–15.50 (10.71)	0.06–1.47 (0.49)	3.63	Positive allometric	Cakic *et al*., (2002)
Corythoichthys flavofasciatus	1	–	13.5	–	3.00	Isometric	Froese and Pauly (2014)
C. schultzi	1	–	12.5	–	3.00	Isometric	
N. ophidion	–	Kiel Bight, Germany	–	–	2.71	Negative allometric	
Lissocampus filum	12	New zeland	6.7–12.5	–	2.89	Negative allometric	
S. pelagicus	17	Cuba	6–21	–	2.66	Negative allometric	
S. typhle	30	Tunisia	15.3 – 30.7 (19.09)	1.6 – 10.75 (2.82)	1.83	Negative allometric	Ben Amor *et al*., 2011
S. abaster	104		7 – 19.8 (9.39)	0.19 – 3.32 (0.56)	2.62	Negative allometric	
S. acus	267		7.1 – 20.7 (9.81)	0.13 – 3.83 (0.65)	2.64	Negative allometric	
N. ophidion	14		15.9 – 16.6 (16.2)	0.4 – 0.56 (0.49)	5.47	Positive allometric	

2013; Venkataraman *et al*., 2013) might have affected their growth pattern. As change in bottom topography and deustruction of associated habitats (e.g. seagrass, algae) may also alter trophic chain (depletion in prey organisms) and flow of energy across the ecosystem (Pauly, 1979) which might affect growth of species.

According to Froese (2006), when '*b'* value, which is the exponent of the arithmetic form of the LWR, is less than 3, then larger specimens of a species have changed their body shape to become more elongated. Similar observations were also reported by Karachle and Stergiou (2012), where streamline fishes ($b < 3$) were reported to grow faster in body length than weight. In the present study, the estimated '*b*' value showed a strong negative allometric relationship in *S. biaculeatus* implying that the weight increases at a slower rate than the body length.

During the present study, the calculated Fulton's condition factor '*K*' for males (0.69 and 0.68 for Palk Bay and Gulf of Mannar specimens, respectively) of *S. biaculeatus* is relatively lower when compared to females (1.08 and 0.84 for Palk Bay and Gulf of Mannar specimens, respectively) and juveniles (1.35 and 1.27 for Palk Bay and Gulf of Mannar specimens, respectively) from both the localities. The condition factor is a quantitative parameter indicating the status of well-being of a fish, and reflects growth and feeding conditions of fish species. Cakic *et al*. (2002) reported '*K*' value of 0.34 ± 0.08 for *S. abaster* population from Danube River. Moreover, Lyons and Dunne (2003) reported significant differences in '*K*' values for different maturity stages of males (immature, 0.37; mature, 0.39; egg-bearing, 0.38 and post-brooding, 0.39) and females (immature, 0.38; and mature, 0.40) in worm pipefish (*Nerophis lumbriciformis*). The '*K*' value is strongly influenced by ecological conditions (Lyons and Dunne, 2003), may vary according to the influences of physiological condition, status of gonad developmental and food availability (Bal and Jones, 1960; Anderson and Neumann, 1996). Male alligator pipefishes are well known for their parental care of broods which incubate in a specially developed open type brood pouch on the ventral surface (Barrows *et al*., 2009). The energy used for developing brood pouch and nutrition of young ones could possibly affect the health of male specimens as observed in the present study.

Chapter 5
Natural diet composition of alligator pipefish, *Syngnathoides biaculeatus* (Bloch, 1785)

5.1. Introduction

The role of natural diet of any fish species has eco-biological implications in sustainable management and conservation of its population (Watanabe *et al.*, 2006; La Mesa *et al.*, 2007; Sara and Sara, 2007; Kitsos *et al.*, 2008). The growth, survival and reproduction for successful propagation of a population are dependent on utilization of energy generated through feeding (Murugan, 2004). Distribution, growth, reproduction, nutritional profile, migration and behaviour of a species are largely influenced by food availability and preferred prey items in its natural habitat (Pfeiler *et al.*, 2000). Fishes are an important source for human nutrition and published literature (Henderson and Tocher 1987; Orban *et al.*, 2007; Velu and Munuswamy 2007; Lin *et al.*, 2008) suggests that the nutritional composition of fishes is strongly affected by their food intake and feeding habits.

The syngnathid fishes are known for their unusual suction feeding with help of their tubular snout, wherein the upper and lower jaws are characterized by the absence of teeth (Foster and Vincent, 2004; Wasswnbergh *et al.*, 2009) which is known as 'pipette feeding' (Muller, 1987) or 'pivot feeding' (de Lussanet and Muller, 2007). These fishes are known to be visual predators, with independently moving eyes. They wait until prey approaches their mouth and with a sudden attack, prey items are rapidly drawn inside the snout with forcible intake of water (Boisseau, 1967; Tipton and Bell, 1988). Syngnathid fishes are also known to be ambush predators (James and Heck, 1994; Bergert and Wainwright, 1997) of amphipods, copepods, fish larvae and other small invertebrates, which fit in their snout gape (Teixeira and Musick, 2001; Woods, 2002; Garcia *et al.*, 2005; Kendrick and Hyndes, 2005; Gurkan *et al.*, 2011a, b; Taskavak *et al.*, 2013).

The ecological and commercial importance of these fishes is well recognized due to their trophic functions and bioactive properties (Vincent, 1996). These fishes mostly inhabit fragile ecosystems and productive coastal areas such as seagrass meadows, corals, mangroves as well as seaweeds (Sreepada *et al.*, 2002) and feed on benthic organisms at the sediment-water interface (Kitsos *et al.*, 2008; Tipton and Bell, 1988). Earlier reports (Tipton, 1987; Tipton and Bell, 1988; Kendrick and Hyndes, 2005;

Kitsos *et al.*, 2008; Storero and Gonzalez, 2008) provide comprehensive information on the feeding ecology of few of these fishes inhabiting seagrass bed.

Syngnathoides biaculeatus is an abundant and the most common member of seagrass fish community of Palk Bay and Gulf of Mannar along the east coast of India (Murugan, 2008). It is evident that there has been no detailed study on the diet composition of alligator pipefish from their natural habitats along the coastal waters of India. Therefore, the present study is the first attempt to evaluate and assess the diet composition of alligator pipefishes collected from the natural habitats of Palk bay.

5.2. Results

The diet composition of 56 individuals including 22 males and 34 females was examined during the present study. The mean total length of selected individuals was 19.28 ± 2.35 cm and wet weight was 5.50 ± 1.05 g. Preliminary examination of fish guts revealed that 5.36 % of guts were empty, 16.07 % moderately full, 37.50 % half-full, 33.93 % quarter-full and 7.14% full, whereas none belonged to the very full gut (100%) category (Fig 5.1). From the above analysis, the Vacuity coefficient index (% I_v) was computed as 5.36 %, while mean Gastro-Somatic index (% *GSI*) was 3.78 ± 1.67 % (males, 3.31 ± 1.50 %; females, 4.09 ± 1.70 %; $P < 0.05$) (Fig. 5.2). The ratio of relative gut length to the total length of *S. biaculeatus* was 0.36 ± 0.03.

Altogether, 10 major groups of prey items were identified from the guts of *S. biaculeatus* (Fig. 5.3, Table 5.1). Numerical abundance of prey items ranged between 68 and 245 (mean ± SD, 124.18 ± 45.82). Although, the mean numbers of prey items in the guts of females (131.64 ± 42.79) were relatively higher than males (112.63 ± 48.90), the inter-sexual differences were insignificant ($P > 0.05$). Among the ten major prey groups, three were non-food items such as sand particles, algal matter and foraminifera. On an average, 124.70 ± 46.84 prey items were recorded from guts of these fishes. Further, among the 21 prey items, 9 taxa were consistently recorded in all guts (Table 5.1). The percentage frequency of occurrence analysis of the prey items revealed 100 % *FO* for copepods and sand particles, followed by amphipods (% *FO* =

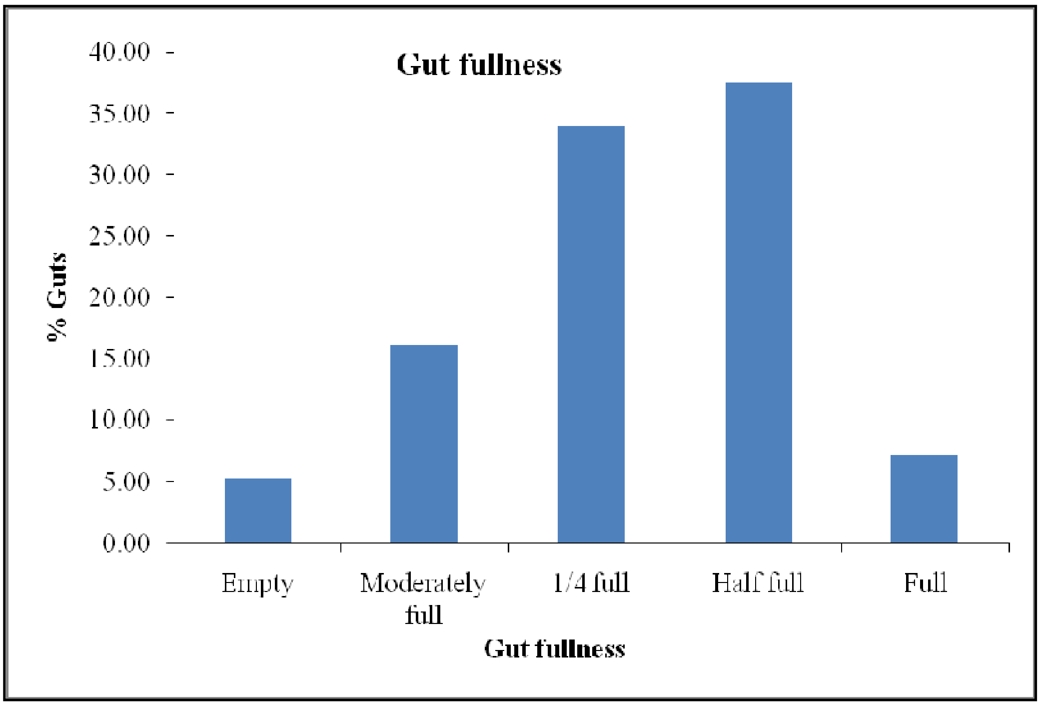

Fig. 5.1. Degree of gut fullness based on six-point scale method.

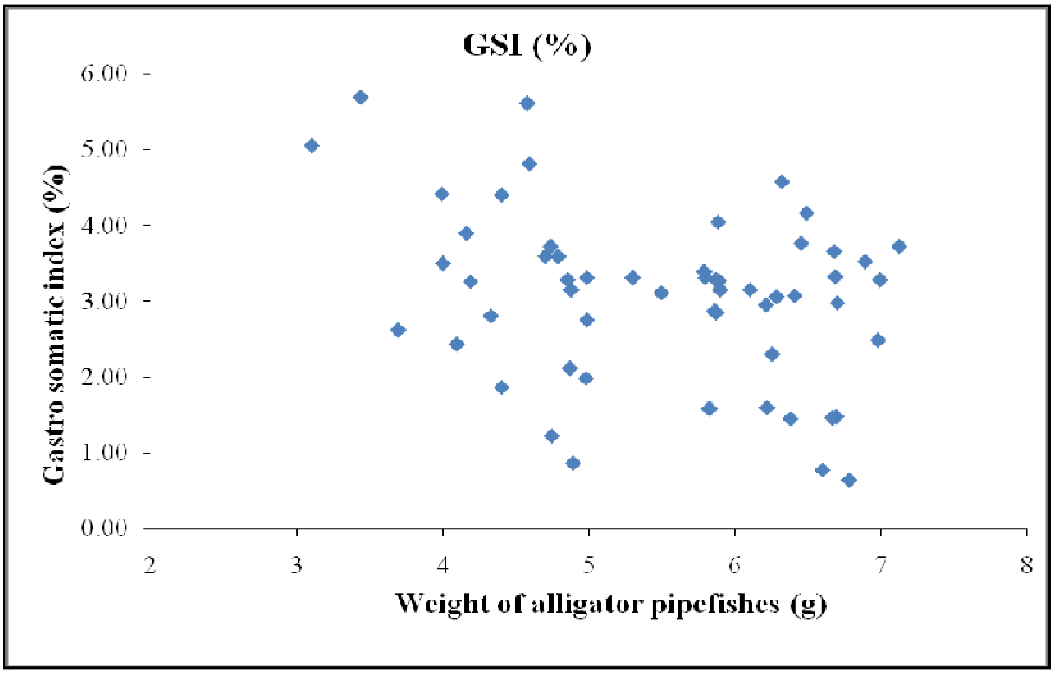

Fig. 5.2. Gastro-somatic index (*%GSI*) of the *S. biaculeatus*.

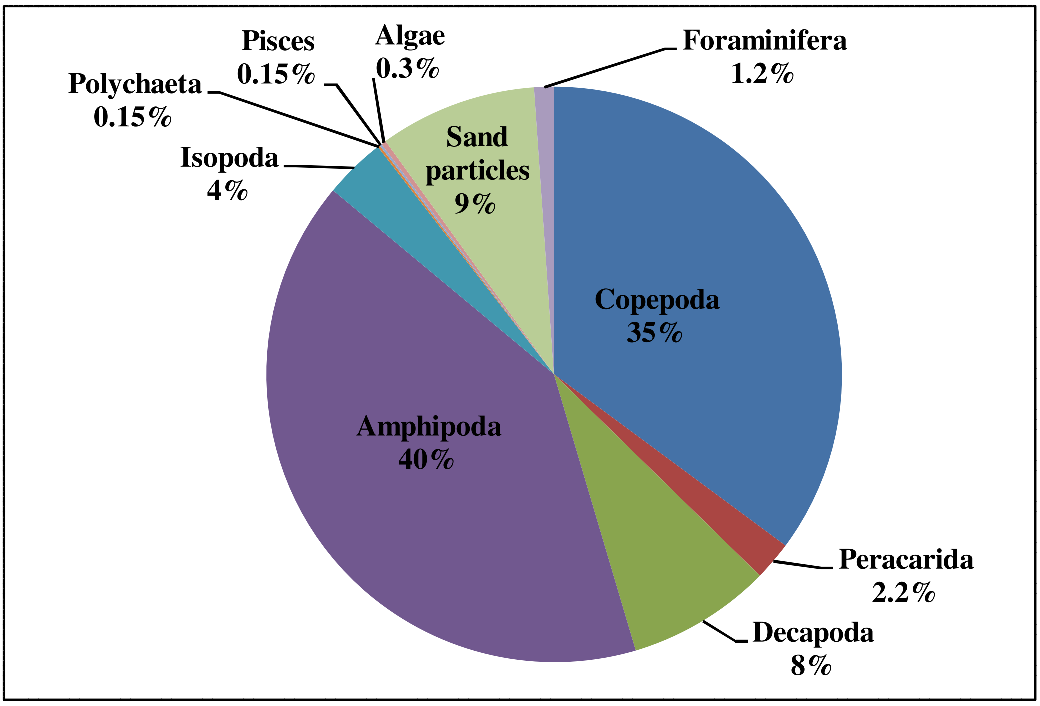

Fig. 5.3. Major groups (%) recorded in the gut content of the*S. biaculeatus*.

Table 5.1. Prey individuals, frequency of occurring (*% FO*) and percentage of prey (*% N*) in gut content of *S. biaculeatus*.

Major group	**Prey items in gut**	**Mean ± SD**	***% FO***	***%N***
Copepoda	*Paracalanus* spp.	16.91 ± 7.26	100.00	13.56
	Acartia spp.	9.66 ± 4.68	100.00	7.75
	Unidentified copepods	17.23 ± 8.59	100.00	13.82
Other Peracarida	Mysids	1.23 ± 1.37	64.15	0.99
	Cumacea	1.52 ± 1.26	77.36	1.22
Decapoda	Megalopa larvae	0.16 ± 0.37	18.87	0.13
	Shrimp larvae	1.98 ± 1.57	79.25	1.59
	Acetes sp.	0.54 ± 0.69	45.28	0.43
	Lucifer spp.	2.36 ± 1.75	83.02	1.89
	Unidentified decapods	5.05 ± 2.14	100.00	4.05
Amphipoda	*Eriopisa* spp.	17.61 ± 7.52	100.00	14.12
	Hornellia spp.	5.45± 3.32	100.00	4.39
	Hyale spp.	3.96± 1.96	100.00	3.19
	Grandidierella spp.	2.38 ± 1.78	96.23	1.90
	Unidentified amphipods	20.91 ± 7.65	100.00	16.77
Isopoda	Isopods	4.32 ± 2.68	92.45	3.47
Annelida	Polychaeta	0.16 ± 0.37	16.98	0.13
Chordata	Pisces	0.16 ± 0.46	13.21	0.13
Algae	Unidentified algae	0.36 ± 0.64	28.30	0.29
Inorganic matter	Sand particles	11.00 ± 4.60	100.00	8.82
Foraminifera	Foraminifera	1.41 ± 1.36	67.92	1.14
Total		124.18 ± 45.82	–	–

99.25), isopods (% *FO* = 86.79), peracarids (% *FO* = 70.76) and decapods (%*FO* = 65.28). From the diet analysis, it is evident that crustaceans (amphipods, copepods and decapods) constituted the major prey category of these fishes. Further quantitative analysis (Table 5.1) revealed that, amphipods were the major prey (40.61 %) followed by copepods (35.13 %), decapods (8.09 %), sand particles (8.82 %), isopods (3.47 %), peracarids (2.21 %) and others (1.68%). Amphipods were represented by *Eriopisa* spp., *Hornellia* spp., *Hyale* spp. and *Grandidierella* spp., while *Paracalanus* spp. and *Acartia* spp. were the major copepod prey. Decapods were represented by crab megalopa larvae, shrimp larvae, *Acetes* sp. and *Lucifer* sp. Among crustaceans, percent number (% *N*) of prey items such as *Eriopisa* sp., *Acartia* spp., unidentified amphipods and copepods were, 13.56, 7.75, 16.77 and 13.82, respectively. Other minor groups observed were polychaetes (% *N* = 0.13), fish larvae (% *N* = 0.13), unidentified algal matter (% *N* = 0.29) and foraminifera (% *N* = 1.14%). Based on the above diet analyses, it could be inferred that crustaceans (~ 90%) were the dominant prey items of *S. biaculeatus*.

5.3. Discussion

Studies pertaining to the eco-biological aspects of syngnathid fishes have greater implications not only in better management of natural resources, but also in conservation through improvements in captive rearing protocols. Most of the recent conservation strategies practiced worldwide highlight the role of captive rearing in restoration of a population. The interaction and survival of these species in natural habitats is constrained by lack of mobility, as these fishes are often seen attached to seagrasses, seaweeds and corals or to any other submerged substratum with their prehensile tail (Foster and Vincent, 2004). Therefore, the source of food material and its availability to these fishes is limited and restricted (Tipton and Bell, 1988). The alligator pipefish, *S. biaculeatus* has been considered to be a permanent resident of seagrass meadows with no reported migration (Dawson, 1985).

In the present study, the natural diet composition of *S. biaculeatus* inhabiting seagrass beds of Palk Bay, southeast coast of India has been assessed and the nature of diet composition in the light of availability of food items has been described. Generally, the gut fullness index of fish provides an insight into the type of prey consumed as well as the process of digestion. The observations made in the present study on the nature of gut fullness suggest that no gut belonged to very full gut (100 %) category whereas, very less percentage of population were found to have empty guts (5.36 %). Review of published literature suggested that, except a study by Gurkan *et al*. (2011a), there are no reports of detailed categorization on the feeding index of pipefish species, thus making it difficult to compare the diet composition. Gurkan *et al*. (2011a) reported less than 10 % empty guts in the Straight-nose pipefish, *Nerophis ophidion*. The large differences in the gut fullness index amongst syngnathid species might be due to the consumption of few larger preys (*Acetes* sp., amphipods) which may fill the gut completely (Garcia *et al*., 2005). For example, Nakamura *et al*. (2003) reported that shrimps (50 %) and fish larvae (25 %) were the major items in guts of *S. biaculeatus* in terms of volume as compared to other smaller crustaceans.

Furthermore, significant gender-specific differences in gut fullness index, with higher values in females, have been reported in few syngnathid fishes (Garcia *et al*., 2005; Kitsos *et al*., 2008). Such inter-sexual differences in feeding index could be attributed to lower feeding activity in males during reproductive period (Oliveira *et al*., 2007).In contrast, no significant inter-sexual differences ($P > 0.05$) in gut fullness index were observed during the present study.

Based on the present observations and comparison with published reports of studies on feeding ecology of syngnathid fishes, it is evident that higher proportion of fishes were reported with empty to half full guts and with low percentage of full guts. In addition to reasons for variations in gut fullness index, the intervening time in fixing the gut contents from original collection time might also reduce the gut fullness due to process of digestion and food assimilation (Woods, 2002). In syngnathid fishes, the time required for food digestion has been estimated to be about 3-4 hours (Foster and Vincent, 2004) or may be even lesser (Murugan *et al*., 2009). Lesser digestion periods might be due to enzymatic activity of bacterial flora associated with gut. In the present study, 16.07 % of guts were moderately full, 37.50 % half-full, while 33.93 % quarter-full, 7.14% full and 5.36 % were empty. Based on various proportions of gut fullness in *S. biaculeatus,* it could be inferred that it is a continuous feeder in the natural habitat.

The Relative Gut Length (RGL) as an indicator of feeding habit of fishes has been widely documented (Horn, 1989; Kramer and Bryant, 1995; Yip *et al*., 2015). Relatively lower RGL values (< 1) are associated with carnivorous fishes, while values of > 3 represent herbivorous or detritivorous type of feeding. In the present study, mean RGL value of *S. biaculeatus* is quite low (0.36 ± 0.03), indicating that it has carnivore nature of feeding. Prey item abundance in syngnathid fish guts is known to vary according to their feeding activity, along with significant inter-sexual differences (Steffe *et al*., 1989; Oliveira *et al*., 2007; Kitsos *et al*., 2008). Altogether, 10 major groups of prey items were identifiable from the guts of *S. biaculeatus* (Table 5.1). Numerical abundance of prey items ranged between 68 and 245 (mean ± SD, 124.18 ± 45.82). Although the mean prey numbers were relatively higher in the guts of females (131.64 ± 42.79) compared to the males (112.63 ± 48.90), the differences; however were insignificant ($P > 0.05$). On the contrary, the present study did not reveal any significant inter-sexual differences in feeding activity. Garcia *et al*. (2005) reported mean prey numbers of 214 and 230, respectively in female and males of the southern pipefish, S*yngnathusfolletti*. Similarly, Taskavak *et al*. (2013) did not report any significant inter-sexual difference in feeding activity of *Syngnathus acus* from Izmir Bay, Turkey. Differential feeding behaviour in the sexes of different pipefish species may be related to the season and reproductive status of these species in the natural environment. However, further studies in this direction are required to corroborate these initial findings.

The analysis of the diet content made in the present study revealed that out of 21 prey items, 9 taxa were consistently observed in individuals of *S.biaculeatus* having prey in their guts (Table 5.1). Copepods and sand particles occurred in the guts of *S. biaculeatus* with 100 % frequency of occurrence followed by amphipods (*%FO* = 99.25) isopods (*%FO* = 86.79), peracarids (*%FO* = 70.76) and decapods (*%FO* = 65.28). From the diet analysis, it is evident that crustaceans (amphipods, copepods and decapods) formed the major diet of *S. biaculeatus* inhabiting seagrass beds of Palk Bay. Amongst all the prey items (Fig. 5.1), amphipods were the major group (40.61%) followed by copepods (35.13%), decapods (8.09%), sand particles (8.82%), isopods (3.47%), peracarids (2.21%) and others (1.68%). Among crustaceans, percent number (*% N*) of prey items such as *Eriopisa* sp., *Acartia* spp., unidentified amphipods and copepods were, 13.56, 7.75, 16.77 and 13.82, respectively. Other minor groups observed were polychaete (%N = 0.13), fish larvae (%N = 0.13), unidentified algal matter (%N = 0.29) and foraminifera (%N = 1.14%) in the guts of *S. biaculeatus*. Based on the numerical abundance, frequency of occurrence and percent number, the prey items of *S. biaculeatus* were predominantly comprised of crustaceans (~90%). Amphipods, gastropods and harpacticoid copepods were also the dominant prey items in the guts of *N. ophidion* from Aegean Sea, Turkey (Gurkan *et al*., 2011a). The variation in occurrence and proportion of prey items in syngnathid fish guts depends upon the type of habitat, prey availability and snout gape. The present study indicated a dominance of various crustacean groups, particularly amphipods and copepods, in *S. biaculeatus* diet. In the diet composition study of Great pipefish, *S. acus*, Taskavak *et al*., (2013) reported that harpacticoid copepods, amphipods, cypris larvae and decapods crustaceans constituted the major prey items in the diet of the great pipefish, *S. acus*. Tipton and Bell (1988) reported the dominance of copepods, amphipods, ostracods, crustacean eggs and caridean shrimps in the diets of Gulf pipefish, *S. scovelli* from Tampa Bay, Florida. Kendrick and Hyndes (2005) reported crustaceans as major prey items in the guts of 12 morphologically diverse syngnathid fishes from seagrass beds of south-western Australia. However, the size and composition of prey items, differed in the individual fishes (Kendrick and Hyndes, 2005). The predominance of crustaceans in the diet of *S. biaculeatus* is in agreement with the diet composition studies of other syngnathid fishes.

The habitat of syngnathid fishes has been reported to play a significant role in feeding behaviour and diet composition (Steffe *et al*., 1989). Teixeira and Vieira (1995) reported that almost entire diet of southern pipefish, *S. folletti* consisted of crustaceans, particularly copepods, amphipods, ostracods and mysids. Interestingly, isopods along with copepods and amphipods were the dominant prey item of same pipefish species inhabiting a Widgeon grass bed in the estuarine zone of the Patos Lagoon, southern Brazil (Garcia *et al*., 2005) which were conspicuously absent in the previous study by Teixeira and Vieira (1995). It is pertinent from the above published reports that notable differences exist in the diet composition of the same species and these are reflected in the different habitats (open waters *v/s* Wadgeon seagrass beds). During the present study, all the individuals of *S. biaculeatus* were collected from seagrass beds of a single locality. Therefore, further studies from different habitats would provide comprehensive idea on diet composition of *S. biaculeatus.*

In this study, sand particles, pieces of algal matter and foraminifera were also observed in guts of *S. biaculeatus* (~ 10%). Earlier studies (Woods, 2002; Murugan, 2004; Garcia *et al*., 2005; Kendrick and Hyndes, 2005; Kitsos *et al*., 2008) from different habitats have also reported the occurrence of sand particles and algae in the guts of pipefishes. As mentioned previously, *S. biaculeatus* is a permanent resident of seagrass beds which are mostly developed on sandy bottom of coastal sea. Therefore, it is possible that while attacking epiphytic prey or feeding on sandy bottoms, sand particles, pieces of seagrass leaves and other algae might have also been unintentionally sucked along with prey organisms.

Availability of food supply in the environment is very important for fish to meet their nutritional requirements in order to grow and survive. Abundance and composition of crustacean species in the ecosystem has been reported to influence the dominant prey items of syngnathid fishes (Storero and Gonzalvez, 2008). In the present study, micro-crustaceans dominated (~ 90%) the prey organisms in *S. biaculeatus* which is similar to other syngnathid fishes. The coastal marine waters along the east coast of India support a greater abundance and rich biodiversity of micro-crustacean species (Venkataraman and Wafar, 2005; Mondal *et al*., 2010). In addition, a variety of prey organisms (larval fishes, shrimps, isopods, cumaceans and mysids) have also been reported from the region in greater abundance (Venkataraman and Wafar, 2005). Therefore, it appears that the natural diet composition of *S. biaculeatus* with dominance of micro-crustaceans is correlated with the composition of prey items in the natural environment. Further studies delineating the differences in prey composition based on habitat, season, snout morphology, etc. are required for better understanding of trophic ecology of pipefishes.

Chapter 6
Biochemical composition of alligator pipefish, *Syngnathoides biaculeatus* (Bloch, 1785)

6.1. Introduction

The application of syngnathid fishes is well recognized as their nutritional profile has wide acceptance in traditional medicine or complementary and alternative medicine for healthcare (WHO, 2002). Seahorses and pipefishes constitute main ingredient in Traditional Chinese Medicine (TCM). They are considered as potential pharmacological mine for various diseases including cancer and impotency (Kumaravel *et al*., 2012). In TCM, seahorses and pipefishes are credited with having a role in increasing and balancing vital energy flows within the body, as well as a curative role for such ailments as impotence, infertility, asthma, high cholesterol, goitre, kidney disorders, and skin afflictions such as severe acne and persistent nodules. They are also reported to facilitate parturition, act as a powerful general tonic and as a potent aphrodisiac (Vincent, 1995a; 1996; Moreau *et al*., 1998; Sreepada *et al*., 2002; Zhang *et al*., 2003; Alves and Rosa, 2006; Shi *et al*., 2006).

The importance of seafood in human diet is now well recognized (WHO, 2002). In human nutrition, polyunsaturated fatty acids (PUFAs), an important ingredient, can help to regulate prostaglandin synthesis and hence induce wound healing (Gibson, 1983; Zuraini *et al*., 2006). It has also positive effects on cardiovascular diseases and various types of cancers (Conner, 1997). At the same time certain amino acids have been reported to play a key role in the process of wound healing (Chyun and Griminger, 1984). The Carbon : Nitrogen ratio in fishes is an indicative of their better physiological condition which is influenced by tissue lipid content and fishes with a balanced composition of protein and lipids reflects higher C:N ratio (Fagan *et al*., 2011). Several naturally occurring antioxidant compounds in TCM sources have been identified as free radical or active oxygen scavengers (Duh, 1998; Pan *et al*., 2007). The antioxidants scavenge free-radicals or potentially harmful reactive oxygen species (ROS) formed during peroxidation and compounds containing oxygen or the chelating capacity of the metals (Kristinsson and Rasco, 2000).

The use of *S. biaculeatus* as an ingredient, popularly known as Hailong in the TCM, has a history of over 600 years (Shi *et al*., 1993; Pogonoski *et al*., 2002). Although biochemical composition and antioxidant properties from seahorse species have been well documented (Hung *et al*., 2008; Lin *et al*., 2008; Lin *et al*., 2009; Qian *et al*., 2008; Qian *et al.*, 2012; Sanaye *et al.*, 2014), the biochemical composition and antioxidant potential of the alligator pipefish, another expensive ingredient in TCM, is lacking. Due to its economic importance in TCM and lack of baseline information on these aspects, the biochemical as well as antioxidant potential of *S. biaculeatus* collected from natural habitat along Palk Bay, southeast coast of India was evaluated in the present study.

6.2. Results

Proximate composition

The proximate analysis of *S. biaculeatus* is presented in Table 6.1. Among proximate components, crude protein,crude lipids, ash and nitrogen-free extracts (% dry weight) were 58.9 ± 2.2, 1.8 ± 0.2, 19.2 ± 2.2 and 20.1 ± 0.45 %, respectively.While, total moisture (% wet weight) was 65.61 ± 0.28%.

Fatty acid profile

The fatty acid composition (% of total fatty acid) of *S. biaculeatus* is shown in Tables 6.2, 6.3 and 6.4. The fatty acid profile of this species consisted of 27 saturated fatty acids (13 straight and 14 branched chained), 28 unsaturated fatty acids (14 monounsaturated and 14 polyunsaturated fatty acids), and nine other fatty acids. The percentages of total saturated and unsaturated fatty acids were 55.41 ± 0.24 and 44.05 ± 0.25 %, respectively. Among all the fatty acids, the percentage of Palmitic acid (C16:0) was the highest (26.93 ± 0.02 %), followed by Oleic acid (15.41 ± 0.01 %), Stearic acid (11.66 ± 0.01%), Lauric acid (6.56 ± 0.02%), Palmitoleic acid (6.25 ± 0.57%), Docosahexaenoic acid (4.55 ± 0.00 %) and Vaccenic acid (4.43 ± 0.00 %). The percentages of monounsaturated fatty acids and polyunsaturated fatty acids were 27.95 ± 0.30% and 16.10 ± 0.09%, respectively (Table 6.5).

Table 6.1. Proximate composition of the *S. biaculeatus*.

Proximate composition	**Value (%)**
Crude protein	58.9 ± 2.2 (dry weight)
Crude lipid	1.8 ± 0.2 (dry weight)
Ash	19.2 ± 2.2 (dry weight)
Moisture	65.61 ± 0.28 (wet weight)
Nitrogen free extracts	20.1 ± 0.45 (dry weight)

Table 6.2.Saturated fatty acids recorded in *S. biaculeatus*.

Commom name	**Fatty acid**	**% to total lipids**
A) Straight chain fatty acids		
Capric acid	C10:0	0.69 ± 0.01
Lauric acid	C12:0	6.56 ± 0.02
Tridecylic acid	C13:0	0.11 ± 0.01
Myristic acid	C14:0	5.53 ± 0.01
Pentadecylic acid	C15:0	0.92 ± 0.00
Palmitic acid	C16:0	26.93 ± 0.02
Margaric acid	C17:0	1.68 ± 0.13
Stearic acid	C18:0	11.66 ± 0.01
Arachidic acid	C20:0	0.43 ± 0.06
Heneicosylic acid	C21:0	0.12 ± 0.01
Behenic acid	C22:0	0.34 ± 0.01
Tricosylic acid	C23:0	0.09 ± 0.00
Lignoceric acid	C24:0	0.31 ± 0.01
Total Straight chain fatty acids		**54.65 ± 0.20**
B) Branched chain fatty acids		
a) Iso series fatty acids		
12-methyltridecanoate	C14:0 iso	0.07 ± 0.00
13-methyltetradecanoate	C15:0 iso	0.30 ± 0.01
9-methyltetradecanoate	C15:1 n-6 iso	0.03 ± 0.07
14-methylpentadecanoate	C16:0 iso	0.23 ± 0.02
15-methylhexadecanoate	C17:0 iso	0.11 ± 0.01
15-methylhexadecanoate	C17:1 n-9 iso	0.49 ± 0.01
17-methyloctadecanote	C19:0 iso	0.07 ± 0.00
b) Anteiso series fatty acids		
10-methyldodecanoate	C13:0 anteiso	0.03 ± 0.07
–	C14:0 anteiso	0.02 ± 0.00
12-methyltetradecanoate	C15:0 anteiso	0.06 ± 0.00
2-methylhexadecanoate	C16:0 anteiso	0.10 ± 0.07
14-methylhexadecanoate	C17:0 anteiso	0.16 ± 0.01
14-methylhexadecanoate	C17:1 n-7 anteiso	0.09 ± 0.00
Total		**0.45 ± 0.02**
Total saturated fatty acids		**55.41 ± 0.24**
18-methylnonadecanote	C20:0 iso	0.15 ± 0.02
Total		**1.44 ± 0.10**

Table 6.3.Unsaturated fatty acids recorded in *S. biaculeatus*.

Commom name	**Fatty acid**	**% to total lipids**
A) Monounsaturated fatty acids		
Myristoleic acid	C14:1 n-9	0.03 ± 0.00
Myristoleic acid	C14:1 n-5	0.04 ± 0.00
Eicosenoic acid	C15:1 n-8	0.03 ± 0.00
Palmitoleic acid	C16:1 n-7	6.25 ± 0.57
Palmitoleic acid	C16:1 n-5	0.18 ± 0.21
–	C17:1 n-8	0.85 ± 0.71
Vaccenic acid	C18:1 n-7	4.43 ± 0.00
–	C18:1 n-5	0.12 ± 0.00
Oleic acid	C18:1 n-9	15.41 ± 0.01
Nonadecenoic acid	C19:1 n-7	0.01 ± 0.00
Gondoic acid	C20:1 n-9	0.45 ± 0.04
–	C20:1 n-8	0.14 ± 0.01
–	C20:1 n-4	0.04 ± 0.00
17-Tetracosenoic acid	C24:1 n-7	0.03 ± 0.00
	Total	**27.95 ± 0.30**
B) Polyunsaturated fatty acids		
Ginkgolic acid	C15:1 n-6	0.02 ± 0.00
–	C15:4 n-3	0.05 ± 0.00
–	C17:1 n-3	0.07 ± 0.00
γ-Linolenic acid	C18:3 n-6	0.38 ± 0.02
Linoleic acid	C18:2 n-6	2.33 ± 0.00
Cis-9-octadecenoic acid	C18:1 n-6	0.10 ± 0.00
Dihomolinoleic acid	C20:2 n-6	0.26 ± 0.01
Arachidonic acid (ARA)	C20:4 n-6	2.66 ± 0.00
Eicosapentaenoic acid (EPA)	C20:5 n-3	2.48 ± 0.01
Docosadienoic acid	C22:2 n-6	0.04 ± 0.00
Adrenic acid	C22:4 n-6	0.95 ± 0.01
Docosapentaenoic acid (DPA)	C22:5 n-3	1.45 ± 0.00
Docosapentaenoic acid	C22:5 n-6	0.79 ± 0.00
Docosahexaenoic acid (DHA)	C22:6 n-3	4.55 ± 0.00
	Total	**16.10 ± 0.09**
	Total unsaturated fatty acids	**44.05 ± 0.25**

Table 6.4.Minor fatty acids found in *S. biaculeatus*.

A) Cyclopropane group		
–	C17:0 n-7 cyclo	0.03 ± 0.00
–	C19:0 n-7 cyclo	0.04 ± 0.00
B) Dimethyl acetal group		
Octadecanal	C18:0 DMA	0.08 ± 0.00
Cis-9-Octadecanal	C18:2 DMA	0.03 ± 0.00
C) 10-methyl ester group		
Methyl hexadecanoate	C16:0 10-methyl	0.22 ± 0.00
Methyl heptadecenoate	C17:0 10-methyl	0.08 ± 0.00
Methyl *cis, cis, cis, cis*-4,7,10,13-hexadecatetraenoate	C18:1 n-7 10-methyl	0.07 ± 0.00
Methyl icosanoate	C20:0 10-methyl	0.03 ± 0.00
D) 2 Hydroxy group		
–	C16:0 2OH	0.02 ± 0.00
	Total	**0.62 ± 0.08**

Table 6.5.Overview of fatty acid composition found in *S. biaculeatus*.

Fatty acid groups	**% to total lipids**
∑ SFA	55.41 ± 0.24
∑ UFA	44.04 ± 0.25
∑ Cyclopropane	00.07 ± 0.00
∑ Dimethyl acetal	00.11 ± 0.00
∑ 10-methyl esters	00.04 ± 0.01
∑ 2 Hydroxy	00.02 ± 0.00
∑MUFA	27.95 ± 0.14
∑PUFA	16.10 ± 0.09
∑ Omega 3 fatty acids (n=3)	08.58 ± 0.00
∑ Omega 6 fatty acids (n=6)	07.52 ± 0.09
∑ Omega 9 fatty acids (n=9)	15.87 ± 0.01
Fatty acid ratios	
PUFA/SFA	0.30 ±0.00
EPA/ARA	0.93 ± 0.00
DHA:EPA	1.84:1
Omega 6: Omega 3	0.88:1

SFA= Saturated fatty acids; UFA= Unsaturated fatty acids; MUFA= Monounsaturated fatty acids; PUFA= Polyunsaturated fatty acids; DHA= Docosahexaenoic acid; EPA= Eicosapentaenoic acid; ARA= Arachidonic acid.

Amino acid profile

The amino acid composition (%) and amino acid profile (total 16 amino acids) of *S. biaculeatus* is shown in Table 6.6. Among all the amino acids (447.43 ± 0.30 mg g^{-1} of dried sample), Glutamic acid/Glutamine (15.27 ± 0.07%), Aspartic acid/Asparagine (10.59 ± 0.02%), Glycine (8.98 ± 0.06%), Arginine (8.29 ± 0.22%), Lysine (7.43 ± 0.26%), Alanine (7.22 ± 0.05%), Leucine (7.04 ± 0.02%) and Proline (6.02 ± 0.01%) formed major constituents. Equal number of essential (38.11 %) and non-essential amino acids (61.89%) were identified from alligator pipefish. Among essential amino acids, Leucine and Lysine contributed 7.04 ± 0.02 and 7.43 ± 0.26 %, respectively. Among non essential amino acids, Glutamic acid/Glutamine (15.27 ± 0.07%) and Aspartic acid/Asparagine (10.59 ± 0.02%) contributed significantly.

Trace element analysis

The concentration (mean ± SD) of nine different trace elements in *S. biaculeatus* collected from its natural environment is presented in Table 6.7. Trace element concentrations in *S. biaculeatus* were generally low and their distribution followed the order, Mg > Fe > Zn > Mn > Cu > Cr > Ni > Hg > Co (Table 6.7). Concentrations of magnesium, iron and zinc were found to be relatively higher than other trace elements and contributed 2215.67 ± 7.57, 121.70 ± 2.10 and 65.48 ± 0.63 µg g^{-1} dry weights of *S. biaculeatus*, respectively.

Carbon:Nitrogen ratio

The measured levels of carbon and nitrogen in *S. biaculeatus* were 50.55 ± 0.04% and 11.57 ± 0.01%, respectively. The calculated C:N ratio was 4.37 ± 0.04.

Table 6.6.Amino acid composition (% mean ± SD) of *S. biaculeatus*.

Amino acids	**Abbreviation**	**Amount in ng 500 ng^{-1} of dried sample**	**Amount in mg g^{-1} of dried sample**	**% to total amino acids**
Alanine	A	16.14 ± 0.09	32.20 ± 0.08	07.22 ± 0.05
Aspartic acid/ Asparagine	B	23.67 ± 0.02	47.33 ± 0.00	10.59 ± 0.02
Arginine	R	18.54 ± 0.48	36.74 ± 0.47	08.29 ± 0.22
Glutamic acid/Glutamine	Z	34.14 ± 0.20	68.41 ± 0.20	15.27 ± 0.07
Glycine	G	20.09 ± 0.15	40.28 ± 0.15	08.98 ± 0.06
Histidine*	H	05.51 ± 0.16	11.13 ± 0.16	02.46 ± 0.07
Isoleucine*	I	10.31 ± 0.02	20.63 ± 0.02	04.61 ± 0.00
Leucine*	L	15.74 ± 0.07	31.53 ± 0.07	07.04 ± 0.02
Lysine*	K	16.62 ± 0.55	32.85 ± 0.55	07.43 ± 0.26
Methionine*	M	06.45 ± 0.07	12.94 ± 0.07	02.88 ± 0.03
Phenylalanine*	F	08.65 ± 0.03	17.33 ± 0.03	03.87 ± 0.01
Proline	P	13.46 ± 0.00	26.92 ± 0.00	06.02 ± 0.01
Serine	S	07.67 ± 0.01	15.32 ± 0.01	03.43 ± 0.00
Threonine*	T	10.20 ± 0.01	20.40 ± 0.01	04.56 ± 0.01
Tyrosine	Y	04.98 ± 0.01	09.96 ± 0.01	02.23 ± 0.00
Valine*	V	11.46 ± 0.70	23.42 ± 0.70	05.12 ± 0.31
TAA		223.619 ± 0.30	447.43 ± 0.30	100
EAA		84.947 ± 0.30	170.22 ± 0.50	38.11
NAA		138.67 ± 0.19	277.20 ± 0.19	61.89
FEAA		107.66 ± 0.30	215.53 ± 0.30	48.10

*Essential Amino Acids (EAA); Total Amino Acid (TAA); Nonessential Amino Acids (NAA); Flavor Enhancing Amino Acids (FEAA).

Table 6.7. Trace element content (µg g^{-1} dry wt; mean ± SD) in *S. biaculeatus* collected from the east coast of India.

Trace elements	**Concentration (µg g^{-1} dry weight)**
Mg	2215.67 ± 7.57
Fe	121.70 ± 2.10
Zn	65.48 ± 0.63
Mn	13.98 ± 0.14
Cu	3.28 ± 0.02
Cr	2.25 ± 0.03
Ni	1.54 ± 0.01
Hg	0.54 ± 0.03
Co	0.08 ± 0.01

Total phenolic content and antioxidant activities

Extraction yield is one of the comparative indicators of antioxidant activity, and was estimated by evaporation of methanol. The extraction yield obtained from *S. biaculeatus* was 30.59 ± 0.57%.

Total phenolic content (TPC)

The estimated TPC measured at 200 µg to 800 µg extract concentrations (Table 6.8) ranged between 19.81 ± 1.10 and 64.04 ± 0.45 mg gallic acid g^{-1} dried extract. In the present investigation, significant positive correlation ($P < 0.05$) between TPC and reducing power ($r = 0.99$), metal chelating activity ($r = 0.98$), DPPH radical scavenging activity ($r = 0.98$), ferric reducing antioxidant power ($r = 0.99$), inhibition of lipid peroxidation ($r = 0.92$) and hydroxyl radical scavenging activity ($r = 0.99$) was observed.

Reducing power (RP)

A significant correlation ($P < 0.05$) between RP and other antioxidant activities indicated the antioxidant potential of *S. biaculeatus*. RP of extracts (0.16 to 0.31 Abs.) was found to be concentration dependent (200–800 µg). Standard ascorbic acid (at 100 µg) showed higher reducing power. The positive correlation between RP and MCA ($r =$ 0.99), DPPH radical scavenging activity ($r = 0.95$), LPX ($r = 0.93$) and HRS ($r = 0.99$) was recorded.

Metal Chelating activity (MCA)

Metal chelating activity (MCA) of *S. biaculeatus* extract varied between 16.59 ± 0.01 and 40.21 ± 0.02% (Table 6.8). A positive high correlation between TPC and MCA ($r = 0.98$) as well as between MCA and lipid peroxidation ($r = 0.95$) was recorded. Furthermore, a high degree of positive correlation ($P < 0.05$) between MCA and other antioxidant activities (RP, $r = 0.99$; DPPH radical scavenging activity, $r =$

Table 6.8.Total phenolic content and antioxidant activities of *S. biaculetaus* at different extract concentrations.

Extract concentration(μg)	**TPC (mg gallic acid g^{-1} dried extract)**	**Reducing power (Absorbance)**	**Metal chelating activity (%)**	**DPPH radical scavenging activity (%)**	**FRAP (mg ASA g^{-1} dried extract)**	**HRS activity (%)**	**LPX Inhibition (%)**
200	19.81 ± 1.10	0.16 ± 0.07	16.59 ± 0.01	6.20 ± 0.21	75.46 ± 0.90	84.89 ± 0.26	54.59 ± 1.5
400	36.09 ± 0.83	0.23 ± 0.05	29.50 ± 0.03	11.56 ± 0.57	100.8 ± 1.00	87.83 ± 0.11	70.34 ± 7.9
800	64.04 ± 0.45	0.31 ± 0.06	40.21 ± 0.02	29.33 ± 1.73	167.25 ± 0.41	91.02 ± 0.01	84.28 ± 3.5

TPC, Total phenolic content; DPPH, 2, 2-diphenyl-1-picrylhydrazyl; FRAP, Ferric reducing antioxidant power; HRS, Hydroxyl radical scavenging activity; LPX, Lipid peroxidation

0.93 and HRS, *r* = 0.99) suggested the greater chelating ability of *S. biaculeatus* extracts.

DPPH radical scavenging activity

DPPH radical scavenging activity measured at extract concentrations from 200 µg to 800 µg varied between 6.20 ± 0.21and 29.33 ± 1.73% (Table 6.8). Standard BHT showed higher activity than extract. A significant positive correlation obtained between DPPH radical scavenging activity and TPC, RP and MCA ($P < 0.05$) reflect the antioxidant capacity of *S. biaculeatus* with free radical scavenging properties.

Ferric Reducing Antioxidant Power (FRAP)

In the present study, FRAP values measured at three different concentrations varied between 75.46 ± 0.90 and 167.25 ± 0.41 mg ASA g^{-1} dried extract (Table 6.8). Relatively high FRAP values and their significant positive correlation ($P < 0.05$) with DPPH radical scavenging activity ($r = 0.99$), LPX ($r = 0.90$) and HRS ($r = 0.97$) indicate the high degree of antioxidant capacity of extracts prepared from *S. biaculeatus*.

Lipid peroxidation (LPX) inhibition assay

The levels of LPX activity recorded at extract concentrations (200 to 800 µg) with standard ascorbic acid ranged between 54.59 ± 1.5 and 84.28 ± 3.5% (Tables 6.8). The positive correlation between LPX and TPC, RP, MCA, DPPH scavenging activity, FRAP and HRS was observed.

Hydroxyl Radical Scavenging (HRS) activity

In the present study, HRS activity of methanolic extract of *S. biaculeatus* wascompared with that of standard ascorbic acid (Table 6.8). HRS activity exhibited by methanolic extracts of *S. biaculeatus*ranged from 84.89 ± 0.26to 91.02 ± 0.01%. Also, significant positive correlation ($P < 0.05$) between HRS activity and otherantioxidant activities demonstrated the efficiency of *S. biaculeatus* extract in stabilizing lipid peroxidation through its hydrogen donating potential.

6.3. Discussion

The Alligator pipefish, *S. biaculeatus* is one of the important species in TCM after seahorses and the only pipefish species traded heavily for TCMs. Although widely used in TCMs, its nutritional profile is not systematically and scientifically studied. During the present study, the biochemical and antioxidant potential of *S, biaculeatus* has been evaluated for the first time from the wild specimens collected from Palk Bay, east coast of India. There is no detailed information about biochemical composition of other pipefish species available, therfore results obtained during present study would be use as baseline for further studies.

Proximate composition

Amongst the proximate principals, crude protein (58.9 ± 2.2% dry wt.) formed the major component in *S.biaculeatus*. This level is marginally lower than the reported values of crude protein in six different species of wild seahorses (Lin *et al.*, 2008). On the other hand, Lin *et al.* (2009) reported crude protein levels of 72.2 ± 2.55 and 68.9 ± 3.4%, respectively in wild and cultured *H. kuda* and marginally higher levels in wild (78.5 ± 4.2%) and cultured (75.6 ± 2.8%) *H. trimaculatus*.The measured levels of other proximate components (moisture, crude lipids and ash) in *S.biaculeatus* are in accordance with Lin *et al.* (2008; 2009). In the case of Snake pipefish, *Entelurus aequoreus* from Bay of Biscay, Spain, proximate levels of crude protein, lipids ash and moisture were 14.7, 1.9 6.8 and 73.5 %, respectively (Spitz *et al.*, 2010). Biochemical composition and nutritional content of wild fishes are often variable and mostly depends upon their feed, geographical conditions, gender and growth stages (Payne and Rippingale, 2000; Lin *et al.*, 2009).

It is well known that the nutritional composition of fish species is strongly affected by their food than other physical parameters (Henderson and Tocher, 1987; Orban *et al.*, 2007; Lin *et al.*, 2008). The natural food of syngnathid fishes mostly consists of small crustaceans such as copepods, amphipods and mysids (Tipton and Bell, 1988;

Garcia *et al*., 2005; personal observation). According to Lin *et al*. (2008), the natural foods of the seahorses may contain high protein (> 75% of dry weight) and low lipid (~3 % of dry weight) which is reflected in their biochemical composition. Proximate composition of major food organisms from Palk Bay, east coast of India has been studied by Murugan *et al*. (2009). Levels of crude protein, lipids and carbohydrates were in the range of 52 and 45 %, 11 and 13 %, 6 and 8%, respectively in amphipods and sergestid shrimps. In a similar study, Perumal *et al*. (2009) reported proximate and amino acid composition of two copepods species, *Acartia spinicauda* and *Oithona similis* from Palk Bay, India. The reported mean levels of crude protein, lipids and carbohydrates in *A. spinicauda* were 67.33–75.45, 12.42–17.81 and 4.01–7.98 %, respectively, and 59.53–69.61, 9.89–15.44 and 3.43–6.59 %, respectively, in *O. similis*. A total of 16 amino acids were also reported from these two copepod species. The diet of *S. biaculeatus* collected from Palk Bay also revealed the dominance of copepods, amphipods, decapods, isopods and other peracarids (Present study, Chapter 5). In view of the above published reports and the observations made in the present study, it is imperative that the nutritional composition of the *S. biaculeatus* collected from its natural habitat is largely influenced by the diet and feeding habits.

Fatty acid profile

In the present study, an analysis of fatty acid composition of *S. biaculeatus* revealed 64 different types of fatty acids represented by 27 saturated fatty acids, SFA (55.41 ± 0.24%), 28 unsaturated fatty acids, UFA (44.05 ± 0.25%) and nine other minor fatty acids (0.62 ± 0.08%). The amount of SFAs quantified during the present study was relatively higher as compared with the values reported earlier by Lin *et al*. (2008; 2009). Generally, high levels of saturated fats are not recommended in foods by Department of Health, UK and the ideal ratio of polyunsaturated fatty acids (PUFA) to SFA should be more than 0.4 and not less than 0.1 for human food consumption (Wood *et al*., 2003). The calculated ratio of PUFA:SFA in *S. biaculeatus* was determined to be 0.3. Monounsaturated fatty acids (MUFA) in *S. biaculeatus* were comparatively higher (27.95 ± 0.30 %) than those values reported in seahorses (Lin *et al*., 2008, 2009), while PUFA (16.10 ± 0.09%) were comparatively lower. According to Mazereeuw *et al*. (2012) and Larsson (2013), Omega 3 fatty acids helphuman metabolism and reducing the risk of cardiovascular diseases, inflammation, developmental disorders and mental health. Omega 3 (n-3), omega 6 (n-6) and omega 9 (n-9) fatty acids found in alligator pipefish were 8.5 8 ± 0.0, 7.52 ± 0.09 and 15.87 ± 0.01%, respectively. Omega 6 to omega 3 fatty acids ratio should be 1:1 for better effect on human health (Simopoulos, 2006). Omega 6 to omega 3 ratio found in alligator pipefish was 0.88:1, which is better than several vegetable oils such as Canola oil (2:1), Soyabean oil (7:1), Olive oil (3-13:1) and corn oil (41:1) (Hibbeln *et al.*, 2006). Among omega 3 fatty acids, Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) are most important for normal human health and these are abundant in marine fishes (Morris *et al.*, 1995; Osman *et al.*, 2001). The sum of DHA and EPA levels in *S. biaculeatus* was 7.02% of total lipids and calculated ratio of DHA: EPA is 1.84:1. Due to relatively high amounts of PUFA (DHA and EPA), alligator pipefishes are most widely used for TCM after seahorses.

Amino acid profile

A total of 16 amino acids were identified in *S. biaculeatus* with an equal proportion of essential (EAA) and non-essential amino acids (NAA). Among the total amino acids (TAA), EAA contributed 38.11 % and NAA the remaining 61.89 %. A comparision of amino acid profile of seahorse species based on published reports (Lin *et al.*, 2008; 2009) and the observations made in the present study suggests that % EAA of *S. biaculeatus* is high. EAAs can be beneficial to improve the immune system and recovery process when consumed by humans (Chyun and Griminger, 1984; Mat Jais *et al.*, 1994; Witte *et al.*, 2002; Wu, 2009). According to studies of Lin *et al.* (2008; 2009) seahorses contain high levels of flavor enhancing amino acids (FAA) such as aspartic acid, glutamic acid, glycine and alanine. Apart from these four mentioned amino acids, phenylalanine and tyrosine also exhibit the same flavor enhancing properties. The present observation indicated that the total FAA in alligator pipefish were 48.20 ± 0.27%, which is comparatively higher than those reported in seahorses by Lin *et al.* (2008; 2009). It has been demonstrated that few amino acids such as Alanine, Arginine, Isoleucine and Proline possess the ability to bind together with Glycine and have ability to form polypeptides, which can trigger tissue re-growth and recovery in humans (Heimann, 1982; Witte *et al.*, 2002).

Trace elements

Trace elements are generally dietary elements that are needed in minute quantities for proper growth, development and physiology of an organism (Bowen, 1966). A total of nine trace elements were reported in the *S. biaculeatus* during the present study. Trace elements from six seahorse species along Chinese coast as well as from cultured and wild seahorses are reported by Lin *et al.* (2008; 2009). The concentrations of Mg and Zn were comparatively higher in *S. biaculeatus* compared to seahorse species, while Mn was marginally lower in *S. biaculeatus*. Other trace elements were more or less similar in concentration in both seahorses and *S. biaculeatus*. According to Lin *et al.* (2008; 2009), Zn and Mn generally play a role in sperm development and also strengthen functioning of kidneys (Xu *et al.*, 2003; Meng *et al.*, 2005) which support the known efficacy of seahorses and pipefishes in TCM. During present study, Fe concentration of *S. biaculeatus* is reported to be 121.70 ± 2.10 µg g^{-1}. This might be helpful in maintaining blood circulation through improved hemoglobin-oxygen carrying capacity of blood in the body as described in TCM (Zhang *et al.*, 1998; Alves and Rosa, 2006; Rosa *et al.*, 2013). Among the toxic heavy metals, Hg was detected from alligator pipefish samples and the measured level of Hg was below the toxic level.

Carbon:Nirogen ratio

Estimation of the C:N ratio has been considered to be an accurate indicator of the condition of fish (Fagan *et al.*, 2011; Martinez-Cardenas *et al.*, 2013). Protein has a C:N ratio close to 3. For instance, it is expected that in a tissue sample from fish in good condition, protein and lipids possess a C:N ratio greater than 3. In contrast, the lipids in starved or poor condition fish are metabolized and the C:N ratio decreases (Westernhagen *et al.*, 1998). In the present study, C:N ratio of *S. biaculeatus* was found to be 4. 37 ± 0.04, indicating optimum physiological state in its natural habitat.These results are in agreement with the rearing experiment study of opossum pipefish, *Microphisbrachyurus*(Martinez-Cardenas *et al.*, 2013), in which they reported C:N ratio of 2.94 ± 0.05 to 3.46 ± 0.1.

Total phenolic content and antioxidant activities

Total phenolic content

Total Phenolic Content values obtained in present study were higher as compared to those reported from wild *H. kuda* (17.43 ± 1.30 mg g^{-1}) by Qian *et al*. (2008). Hydrogen or electron donation, metal ion chelation, neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides are the reactions exhibited by phenolic compounds during free radical scavenging activities (Osawa, 1994; Rice-Evans *et al*., 1996). A good correlation between TPC from different sources and their antioxidant potential has been documented by several workers (Bakkalbasi *et al*., 2005; Aliakbarian *et al*., 2008; 2009; Ben Hamissa *et al*., 2012). A comparison of TPC levels recorded in the present study with the above cited literature reflected that *S. biaculeatus* possess high degree of antioxidant property.

Reducing Power

Reducing capacity of an extract may serve as an indicator for potential antioxidant activity. The present results agree with RP values reported by Qian *et al*. (2008) for *H. kuda*. However, relatively higher RP values were reported by Hung *et al*. (2008) for *H. kuda,* which could be attributed to higher concentration of extract (40 mg ml^{-1}). Reducing agents can reduce the oxidized intermediates such as metal ions during lipid peroxidation process (Chanda and Dave, 2009). The positive correlation between RP and other activities indicate the greater ability of *S. biaculeatus* extracts to provide hydrogen ions for electrons to reduce metal ions and their subsequent involvement in scavenging of free radicals.

Metal Chelating Activity

During present study, MCA of *S. biaculeatus* extract varied between 16.59 ± 0.01 and 40.21 ± 0.02 %. MCA plays an important role in antioxidant mechanisms due to its capability to reduce the concentration of the catalyzing transition metal in lipid peroxidation process (Duh *et al*., 1999). In addition, it has been reported that some phenolic compounds exhibit antioxidant activity through the chelation of metal ions (Zhao *et al*., 2008). However it is also worth to note that Hung *et al*. (2008) reported low MCA (4.89 ± 0.53 to 8.35 ± 0.05 % at 40 mg ml^{-1} extract concentration) in cultured *H. kuda*.

DPPH radical scavenging activity

DPPH radical scavenging activity observed in the present investigation was found to be relatively lower than reported for seahorses (Qian *et al*., 2008; Hung *et al*., 2008). DPPH is a stable free radical and accepts an electron or hydrogen radical to develop into a stable diamagnetic molecule (Duh *et al*., 1999). Therefore, DPPH is often used as a substrate to evaluate the degree of activity of natural antioxidants. Radical scavenging activity is also attributed to phenolic content of the extract (Kim *et al*., 2002; Lee and Seo, 2006). The levels of DPPH radical scavenging activity exhibited by extracts often reflect their ability to provide hydrogen ion and/or electrons to bind or to capture metal ions (Griffin and Bhagooli, 2004).

Ferric reducing antioxidant power

FRAP assay reduces ferric complex (Fe^{+3}-TPTZ) to ferrous (Fe^{+2}-TPTZ) and measures the potentiality of biological antioxidants (Griffin and Bhagooli, 2004). In the present study, FRAP values measured at three different concentrations varied between 75.46 ± 0.90 and 167.25 ± 0.41 mg ASA g^{-1} dried extract. Relatively high FRAP values and their significant positive correlation ($P < 0.05$) with DPPH radical scavenging activity ($r = 0.99$), LPX ($r = 0.91$) and HRS ($r = 0.98$) indicate the high degree of antioxidant capacity of *S. biaculeatus* extracts.

Lipid peroxidation inhibition assay

According to Cheng *et al*. (2003) phenolic compounds exert their protective action in lipid peroxidation by scavenging the lipid derived radicals ($R^{\cdot}$, $RO^{\cdot}$ or $ROO^{\cdot}$). Lipids upon reaction with free radicals undergo the highly damaging chain reaction of lipid peroxidation leading to both direct and indirect effects (Devasagayam *et al*., 2004). Results obtained in the present investigation indicated that methanolic extract of *S. biaculeatus* extract has a capacity to minimize lipid peroxidation by inhibition of free radicals and quenching of hydroxyl radicals in biological cells.

Hydroxyl radical scavenging activity

Amongst all the oxidative radicals, hydroxyl radicals are the most reactive free radicals formed in biological systems, which can almost react with all the substances in the living cells and induce severe damage to the cells (Halliwell and Gutteridge, 1990). Levels of activity recorded in the present study are relatively higher than those reported for *H. kuda* (Qian *et al*., 2008). Significant positive correlation between HRS activity and TPC (r = 0.99) was found in the present study. It has been reported that the phenolic compounds donate electrons to hydroxyl radical thus neutralizing it to water (Rabiei *et al*., 2012).

It is pertinent to note that much of the biochemical parameters studied in the present study provide baseline data for the *S. biaculeatus*. Due to non availability of earlier published literature on these aspects of pipefishes, it has been difficult to compare the data in a more comprehensive manner. Hence, it is mentioned that the data presented here need to be used with care and to be treated as baseline data.

Chapter 7

Captive rearing trials and reproductive behavior of alligator pipefishes, *Syngnathoides biaculeatus* (Bloch, 1785)

7.1. Introduction

A better insight on the life history parameters and culture techniques of a species helps in large-scale production as well as in the conservation and management of depleting fish stocks in the wild. Captive breeding and rearing have been recognized as a long-term solution for sustaining the seahorse trade while minimizing wild collection (Sreepada *et al.*, 2002). Fishery scientists are concerned with aspects of reproductive processes like mating, time of spawning, recruitment, size or age at first maturity, sex ratio, fecundity, etc., which are the baseline information for selecting a species for commercial aquaculture, developing controlled breeding programs and establishing low cost hatcheries (Murugan *et al.*, 2009; Koldeway and Martin-Smith, 2010). These parameters are also considered as vital for stock enhancement programs to protect vulnerable species such as the syngnathids from population decline (Wong and Benzie, 2003; Koldeway and Martin-Smith, 2010).

Among Syngnathids, the female deposits unfertilized eggs into the male's specialized ventral incubating area located on the abdomen (Gastrophori) or tail (Urophori), whose form varies among the pipefish genera (Herald, 1959). Some species attach eggs simply to the skin of males without any protecting plates or covering membranes, while others deposit eggs into a brood pouch (Wilson *et al.*, 2003). Eggs are fertilized within the brood pouch ensuring paternity of the brooding male (McCoy *et al.*, 2001) and the embryos are nourished, osmo-regulated, aerated and protected during the lengthy incubation period (Carcupino *et al.*, 1997; 2002). Many researchers have studied the reproductive biology (maturity, gonad development) and mating patterns (courtship behavior, spawning) of few pipefish species (Berglund *et al.*, 1986; Campbell and Able, 1998; Monteiro *et al.*, 2001; Takahashi *et al.*, 2003; Barrows *et al.*, 2009; Gurkan *et al.*, 2009) in captive conditions. Also, extensive literature is available on the structure of gonads, importance and functions of brood pouch, mating patterns and sex reversal (Fuller and Berglund, 1996; Jones and Avise, 2001; Carcupino *et al.*, 2002; Wilson *et al.*, 2003; Kvarnemo and Simmons, 2004; Monteiro *et al.*, 2005; Van Look *et al.*, 2007). Although exhaustive literature is now available on the reproductive biology and mating pattern of syngnathid fishes, it is pertinent to note that such techniques and their applications may vary in accordance with biogeography, climate and biological conditions.

Reef systems are unique and known to support a variety of marine flora and fauna. However, over-fishing of valuable reef organisms has led to a situation wherein many reefs are left with very few adults to replenish stocks and/or the breeding populations of several species have been eliminated. These fish catches are primarily driven by economic forces that eventually overwhelm slowly replenishing stocks (Ban and Alder, 2008). In few cases, specific stocks have been so severely overexploited, that they are now listed as endangered. Global trade in marine ornamental fishes is a major international industry involving an annual catch of 14–30 million fishes (Woods, 2001). The effort to reduce the fishing pressure from coral reef habitats is being carried out by way of providing alternative livelihood options to the dependent communities. Due to lack of captive breeding techniques and accompanying high price of farmed fishes, the pressure on wild fish stocks has been increasing.

India has a long history of trade in pipefishes and fishery for these species started in the southeast coast of India, particularly Tamil Nadu in the year 1992 owing to burgeoning demand as well as due to a reduction in sea cucumber catch (Marichamy *et al*., 1993). Syngnathids are also frequently landed as by-catch of various fishing operations throughout the world (Vincent, 1990; Salin *et al*., 2005; Murugan *et al*., 2008). Hence, the captive breeding and culture of syngnathids gained considerable importance to meet the global demand (Vincent, 1996; Woods and Valentino, 2003; Job *et al*., 2002), and a number of species are now successfully reared (Forteath, 1996; Giwojna and Giwojna, 1999; Lu *et al*., 2002). However, large commercial scale culture was technically challenging due to low growth, low reproduction and poor juvenile survival in the early rearing phase and problems in providing sufficient high quality feed (Vincent, 1996; Payne and Rippingale, 2000, Koldeway and Martin-Smith, 2010). Although, the potential for culture of pipefishes has been discussed, their culture under captive conditions has not been attempted (Dhanya, 2008).

The development of an appropriate rearing protocol is the major problem in raising marine fishes in captivity (Ziemann, 2001). Two key bottlenecks have been indentified to limit the expansion of marine fish aquaculture industry, the first being the control of maturation and spawning to ensure a constant supply of seeds and the identification of appropriate live food items for larval first feeding (Ostrowski and Laidley, 2001).

Few studies have been carried out on some aspects of reproductive behaviour and life history of *S. biaculeatus* using wild caught pregnant males (Takahashi *et al*., 2003; Dhanya *et al*., 2004; Dhanya, 2008; Barrows *et al*., 2009). More comprehensive information on the reproductive biology of *S. biaculeatus* from its natural habitat (Palk bay, east coast of India) is available through Dhanya (2008). Therefore, the objective of this study is limited to provide information on captive rearing of *S. biaculeatus* adults and juveniles with available feeds and conditions along the west coast of India. It is expected that the results of this study would help in enhancing our knowledge towards captive breeding of *S. biaculeatus,* which is important to optimize reproduction and culture of this species.

7.2. Results

Captive rearing of adult *S. biaculeatus*

No mortality in captive reared adult alligator pipefishes was observed during rearing period of 12 months.

Courtship behavior and mating

Selection of partner was first observed with the courtship behaviour in *S. biaculeatus,* initiated after 20 to 30 days after rearing in all glass breeding tanks. Courtship behaviour generally started in the morning hours and was observed to last for 4-5 hours till noon, less activity was observed during after noon hours. During initiation of courtship, females were often found to display a white zigzag pattern on the undersurface of the abdomen in vertical position, by holding rope holdfast with their prehensile tail in an 'S' shape posture. Head and snout were observed to be in slightly downward position. The males responded by swimming close to the females facing their ventral abdominal surface (displaying brooding area) and in 'head down' position. The colour of courting males changed from light green to dark green with brown shades at the side edges of the body, which is often called as 'Brightening'. Along the dorsal and lateral (side) surfaces of alligator pipefishes, white patches containing red reticulate patterns were developed. This colouration was also observed on tail ridges in almost every ring as large dots. Both male and female shook or vibrated their body, dorsal fin for two to four seconds, close to each other, which is called as 'Quivering'. This action was repeated by males and females swimming side by side (Promenading), until the female selected a partner from the group of males.

On the next morning, daily greetings were observed, wherein the female approached the male and displayed the same courtship behaviour as described earlier. The males also joined them with “head down” and “tail up” positions and both fishes were observed with their ventral abdominal surfaces facing each other and quivering. The courtship behaviour was observed to last for two to three days with the forming of male-female pairs. Changes in female abdomen were observed as bulging of abdomen as a result of fully developed ovaries and protruding genital papilla (a tube for transfer of eggs). During the mating, each pair was observed to rise several times up the water column facing one another (Dry run). After several attempts, during the final copulatory rise, the female laid or attached eggs on the male ventral abdominal region and subsequently, the pair got separated. Both the males and the females slowly sank down the water column, and held on to the holdfast. Few eggs (4 ± 2 nos.) were found at the tank bottom, possibly dropped during mating.

All matured eggs were first hydrated in female ovaries just before spawning. The average size of hydrated eggs was 2.1 ± 0.3 mm, and the eggs were found to be spherical in shape with transparent white colour (Plate 7.1A). Subsequently, fertilization occurred in the male’s brood pouch and further embryonic development occurred in the open type brood pouch. Every egg got attached along with its separate chamber to the ventral abdominal surface.

Fecundity and gestation

Transparent white eggs slowly developed and became light to dark brown at the end of gestation. Completely developed embryos could be easily observed inside the eggs (Plate 7.1B). Average gestation period for 14 successful mating and spawning attempts were observed to be 18 ± 2 days at 27 ± 1.5 ºC. Average numbers (from 14 attempts) of eggs (dropped eggs as well as attached eggs) laid by each female was 155 ± 44. The maximum eggs laid by female were 215 during first mating attempt, while the lowest number of 64 eggs was recorded during second mating.

Spawning

Batch spawning mode was observed in the case of *S. biaculeatus*. The release of juveniles was high during morning hours with backward movement of male body, horizontally at the surface of the tank water. Maximum duration recorded for the release of all juveniles was 3 days, while release of all juveniles in a single day was also observed. The time period between release of juveniles varied between 2 to 30 minutes when whole brood was released on single day.

Egg development was not uniform and hatching was observed to start from the outer surface and then progressing inwards. The sizes of new born juveniles were 1.9 to 2.0 ± 0.08 cm and wet weight were 70.07 ± 0.06 mg. Juvenile pipefishes were miniature forms of adult pipefishes and differed mostly in having a more streamlined cylindrical body, and brown color with no yolk (Plate 7.2). After their birth, all juveniles were able to swim freely in the tank and observed to hold each other's tail in group. They were also observed to accept live prey immediately after their birth.

Inter mating duration

A total of nine pairs were reared during the rearing period of 12 months in captivity. Successful mating and spawning was observed for 14 times during 12 months. Out of the nine pairs, two pairs bred thrice, two pairs twice and four pairs bred only once, while one unsuccessful mating was observed. Average inter-mating period (duration between two matings) was observed to be 21 ± 2 days. All females were observed to be mating with their respective partners, which indicated monogamous mating pattern of alligator pipefishes, except one failed attempt of mating between one paired female with another non-paired male.

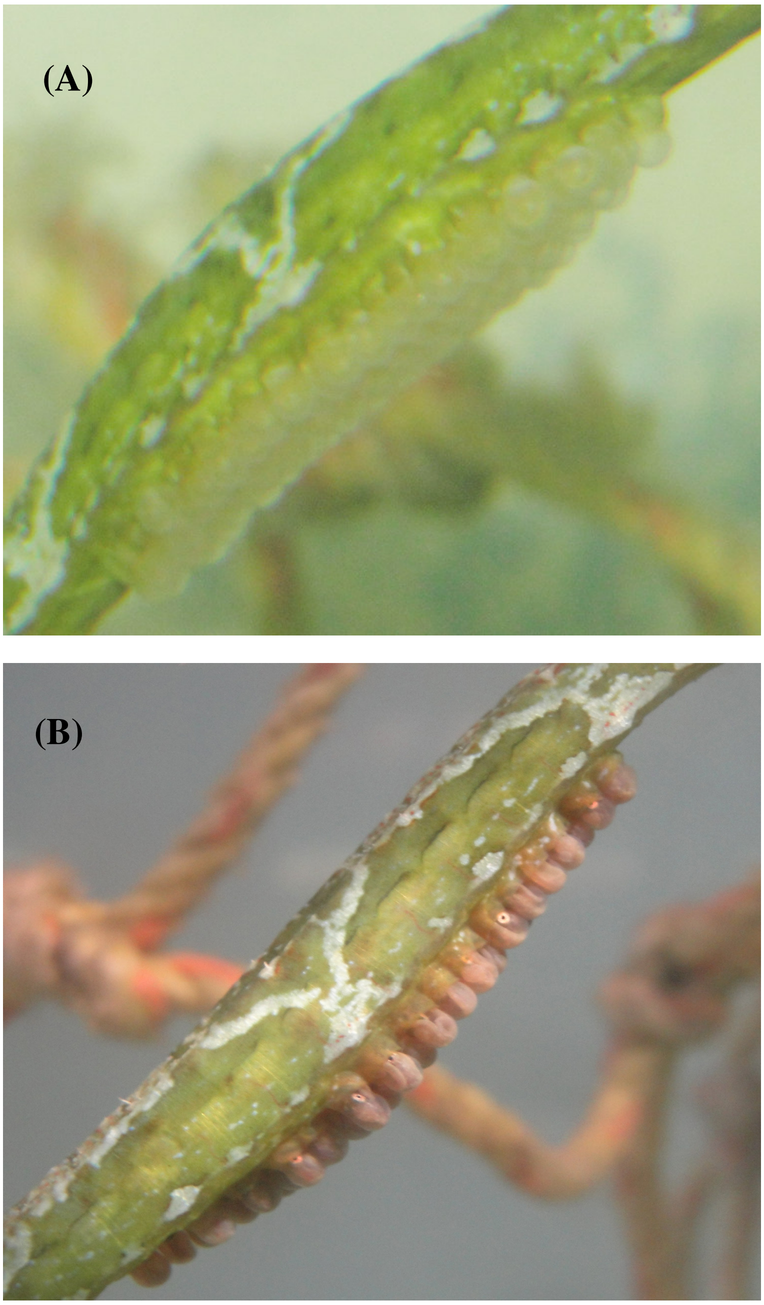

Plate 7.1.(A) White patches on dorsal and lateral sides of male fishes indicatingthe presence of attached eggs in open type brood pouch (on ventral side) of male specimens of *S. biaculeatus*. (B) Developed embryo after 16 days of gestation period with attached eggs.

Plate 7.2.New born juveniles of *S. biaculeatus* with brown colour and grouping of juveniles.

External gas bubble disease (EGBD)

The prevention of gas bubble disease consists of avoiding sudden differences of gas tension and hence the super-saturation of gases associated with it. Acetazolamide (trade name 'Diamox®') is a non-bacteriostatic sulphonamide. After 3-4 days of application, EGBD was observed to be cured and gas bubbles formed under the skin had disappeared.

Captive rearing of *S. biaculeatus* juveniles

Experiment I

Effect of marine zooplankton and *Artemia* nauplii on growth and survival of *S. biaculeatus* juveniles

The percentage average length gain (%) of juveniles fed with zooplankton and *Artemia* nauplii was 228.20 ± 1.62 and 224.07 ± 1.09, respectively and the corresponding percentage average weight gain (%) was 135.40 ± 1.08 and 130.14 ± 0.49, respectively after 20 days of rearing (Table 7.1). The percentage average Specific Growth Rate (SGR) in terms of weight of juveniles, fed with zooplankton and *Artemia* nauplii was 353.29 ±3 and 341.60 ± 0.16, respectively after 20 days (Table 7.1). No mortality was observed during the rearing period in juveniles fed with zooplankton, while the survival rate of 90% was recorded in juveniles fed with *Artemia* nauplii (Table 7.2). The *t*-test revealed that the average length gain (%), weight gain (%), SGR (%) and survival (%) of the juveniles fed with zooplankton was significantly higher ($P < 0.05$) than those fed with *Artemia* nauplii.

Table 7.1. Growth profile of *S. biaculeatus* juveniles fed on zooplankton and *Artemia* nauplii.

Particulars	Diet	
	Zooplankton	***Artemia* nauplii**
Initial length (mm)	19.53 ± 0.09	19.50 ± 0.02
Initial weight (mg)	70.07 ± 0.06	70.07 ± 0.12
Length after 20 days (Mean ± SD)	64.11±0.06	63.20±0.05
Weight after 20 days (Mean ± SD)	144.87 ± 0.45	141.49 ± 0.05
Length gain (%)	228.20 ± 1.62	224.07 ± 1.09
Weight gain (%)	135.40 ± 1.08	130.14 ± 0.49
SGR (%)	476.33± 0.30	473.98± 0.05
Survival (%)	100	90

Table 7.2.*t*-Test of length gain (%), weight gain (%) and SGR (%) of *S. biaculeatus*juveniles fed on zooplankton and *Artemia* nauplii.

Growth parameters	**Zooplankton**	***Artemia* nauplii**	***t*-Test significance**
Length gain (%)	228.20 ± 1.62	224.07 ± 1.09	P<0.05
Weight gain (%)	135.40 ± 1.08	130.14 ± 0.49	P<0.05
SGR (%)	476.33± 0.30	473.98± 0.05	P<0.05
Survival (%)	100	90 ± 5	P<0.05

Experiment II

Effect of three different live food organisms on growth and survival of *S. biaculeatus* juveniles

The average length gain of juveniles fed on amphipods, mysids and adult *Artemia* was 167.67 ± 0.14, 166.08 ± 0.30 and 159.75 ± 0.3%, respectively for rearing duration of 90 days (Table 7.3). ANOVA showed significant difference ($P < 0.05$) in length gain of juveniles fed with different live food organisms (Table 7.4A, B). The Newman-Keuls multiple comparison test showed that the average length gain of the juveniles fed on amphipods was significantly higher ($P < 0.05$) from those fed with mysids and adult *Artemia*.

The average weight gain of juveniles fed on amphipods, mysids and adult *Artemia* was 1742.76 ± 1.60, 1741.20 ± 1.28 and 1726.25 ± 0.53 %, respectively in rearing duration of 90 days (Table 7.3). ANOVA showed significant difference ($P < 0.05$) in weight gain of *S. biaculeatus* juveniles fed on different live food organisms (Table 7.5A, B). The Newman-Keuls multiple comparison test showed that the average weight gain of the juveniles fed with amphipods was significantly higher ($P < 0.05$) from those fed with *Artemia*, while there was no significant difference ($P > 0.05$) between juveniles fed with amphipods and mysids.

The average SGR (%) of juveniles fed with amphipods, mysids and adult *Artemia* was 782.93 ± 0.03, 782.83 ± 0.02 and 781.99 ± 0.02 % respectively in rearing duration of 90 days (Table 7.3). ANOVA showed significant difference ($P < 0.05$) in SGR of juveniles fed with different live food organisms (Table 7.6A, B). The Newman-Keuls multiple comparison test revealed that the average SGR of the juveniles fed with amphipods was significantly higher ($P < 0.05$) from juveniles fed with mysids and adult *Artemia*.

The average survival (%) of juveniles fed with amphipods, mysids and adult *Artemia* was 93.33 ± 2.89, 86.67 ± 2.89 and 85 ± 0 % respectively in rearing duration of 90 days (Table 7.3). ANOVA showed significant difference ($P < 0.05$) in survival %

Table 7.3. Growth and survival of *S. biaculeatus* juveniles fed on different live food organisms.

Particulars	**Treatments**		
	amphipods	**Mysids**	**Adult*Artemia***
Initial length (mm)	64.10 ± 0.04	64.08 ± 0.04	64.12 ± 0.08
Initial weight (mg)	144.12 ± 0.08	144.10 ± 0.08	144.06 ± 0.03
Length after 90 days (Mean ± SD)	171.59 ± 0.15	170.69 ± 0.14	166.56 ± 0.08
Weight after 90 days (Mean ± SD)	2655.81 ± 0.85	2653.08 ± 0.40	2630.90 ± 0.80
Gain in length (mm)	107.48 ± 0.12	106.61 ± 0.15	102.44 ± 0.09
Gain in weight (mg)	2511.69 ± 0.93	2508.99 ± 0.46	2486.84 ± 0.48
Length gain (%)	167.67 ± 0.14	166.38 ± 0.30	159.75 ± 0.30
Weight gain (%)	1742.76 ± 1.60	1741.20 ± 1.28	1726.25 ± 0.53
SGR (%)	782.93± 0.03	782.83± 0.02	781.99± 0.02
Survival (%)	93.33± 2.89	86.67± 2.89	85.00± 0.00

Table 7.4A. Analysis of variance (ANOVA) of length gain (%) of *S. biaculeatus* juveniles fed with different live food organisms.

Source of Variation	**Degrees of Freedom**	**Sum of Square**	**Mean Square**	**F value**
Between Groups	2	108.40	54.19	789.60
Within Groups	6	0.41	0.07	
Total	8	108.80	**-**	

Table 7.4B. Newman-Keuls multiple comparison test for length gain (%).

Comparison	**Mean difference**	**Newman-Keuls multiple comparison test**	**Conclusion**
amphipods*vs*mysids	– 1.29	Significant	$P < 0.05$
amphipods*vsArtemia***	– 7.92	Significant	$P < 0.05$
mysids*vsArtemia***	– 6.62	Significant	$P < 0.05$

Table 7.5A. Analysis of variance (ANOVA) of weight gain (%) of *S. biaculeatus*juveniles fed with different live food organisms.

Source of Variation	**Degrees of Freedom**	**Sum of Square**	**Mean Square**	**F value**
Between Groups	2	498.40	249.2	166.30
Within Groups	6	8.99	1.49	
Total	8	507.40	**-**	

Table 7.5B. Newman-Keuls multiple comparison test for weight gain (%).

Comparison	**Mean difference**	**Newman-Keuls multiple comparison test**	**Conclusion**
amphipods*vs*mysids	–1.55	Non Significant	$P > 0.05$
amphipods*vsArtemia*	–16.51	Significant	$P < 0.05$
mysids*vsArtemia*	–14.95	Significant	$P < 0.05$

Table 7.6A. Analysis of variance (ANOVA) of SGR (%) of *S. biaculeatus* juveniles fed with different live food organisms.

Source of Variation	Degrees of Freedom	Sum of Square	Mean Square	F value
Between Groups	2	1.60	0.80	1286
Within Groups	6	0.00	0.0006	
Total	8	1.60	-	

Table 7.6B.Newman-Keuls multiple comparison test for SGR (%).

Comparison	Mean difference	Newman-Keuls multiple comparison test	Conclusion
amphipods*vs*mysids	–0.10	Significant	P<0.05
amphipods*vsArtemia*	–0.94	Significant	P<0.05
mysids*vsArtemia*	–0.83	Significant	P<0.05

Table 7.7A. Analysis of variance (ANOVA) of Survival (%) of *S. biaculeatus* juveniles fed with different live food organisms.

Source of Variation	Degrees of Freedom	Sum of Square	Mean Square	F value
Between Groups	2	116.70	58.33	10.50
Within Groups	6	33.33	5.55	
Total	8	150	-	

Table 7.7B.Newman-Keuls multiple comparison test for survival (%).

Comparison	Mean difference	Newman-Keuls multiple comparison test	Conclusion
amphipods*vs*mysids	–6.66	Significant	*P*<0.05
amphipods*vsArtemia*	–8.33	Significant	*P*<0.05
mysids*vsArtemia*	–1.66	Non significant	*P*>0.05

of juveniles fed with different live food organisms (Table 7.7A, B). The Newman-Keuls multiple comparison test revealed that the average survival of the juveniles fed with amphipods was significantly higher ($P < 0.05$) from mysids and adult *Artemia*. There was no significant difference observed between mysids and adult *Artemia* ($P > 0.05$).

Growth profile

Growth profile of *S. biaculeatus* juveniles over a period of 110 days (combined for both experiments) is shown in Figure 7.1 and 7.2. The *S. biaculeatus* juveniles attained a mean total length of 64.1, 85.8, 113.7, 135.4, 156.8, 169.61 mm (Fig. 7.1); while the mean weights attained were 144.09, 235.563, 562.67, 1186.72, 1838.8, 2646.6 mg in 20, 30, 50, 70, 90 and 110 days, respectively (Fig. 7.2). Average growth rates (total length day^{-1}) obtained during 110 days rearing period were 2.28, 0.34, 0.33, 0.19, 0.16 and 0.08 for 20, 30, 50, 70, 90 and 110 days, respectively, suggesting high SGR values during the initial period of growth. The rate of increase in terms of weight up to 50 days was low and after 50 days increased in weight was observed.

Feeding rate

Feeding rate was observed to be higher in the morning and evening, and lesser at noon. Initially, feeding rate at 0800, 1200 and 1800 hours was 11.6 ± 1.70, 4.6 ± 0.50 and 14 ± 0.70 $min^{-1}$for zooplankton and 9 ± 0.70, 3.4 ± 0.50 and 11.2 ± 1.60 $min^{-1}$for *Artemia* nauplii, respectively. Feeding rate observed to be time-dependent and increased as juveniles grew in size. Feeding rates of 110 days old juveniles for three different live good organisms at 0800, 1200 and 1800 hours were 8.4 ± 0.89, 5.6 ± 1.14 and 8.6 ± 1.10 amphipods min^{-1}; 8.2 ± 0.84, 5.6 ± 0.89 and 8.4 ± 0.89 mysids min^{-1}, and 8.2 ± 0.84, 5.2 ± 0.84 and 8.2 ± 0.84 *Artemia* min^{-1}, respectively. No feeding was observed atdark hours during rearing of *S. biaculeatus* juveniles.

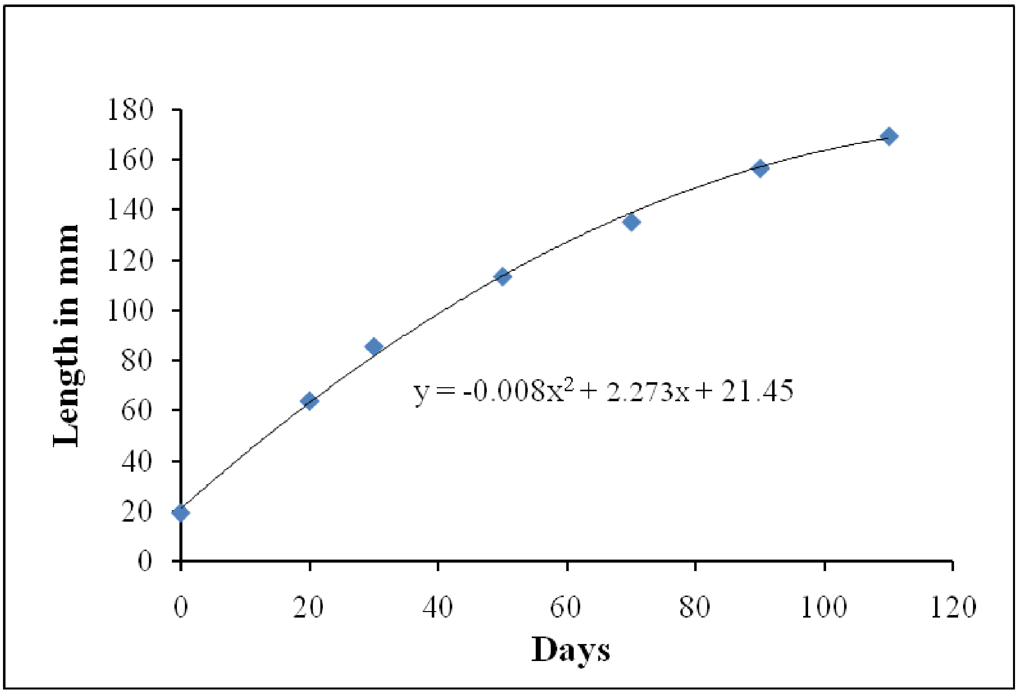

Fig. 7.1. Growth profile of *S. biaculeatus*juveniles in terms of total length duringrearing period.

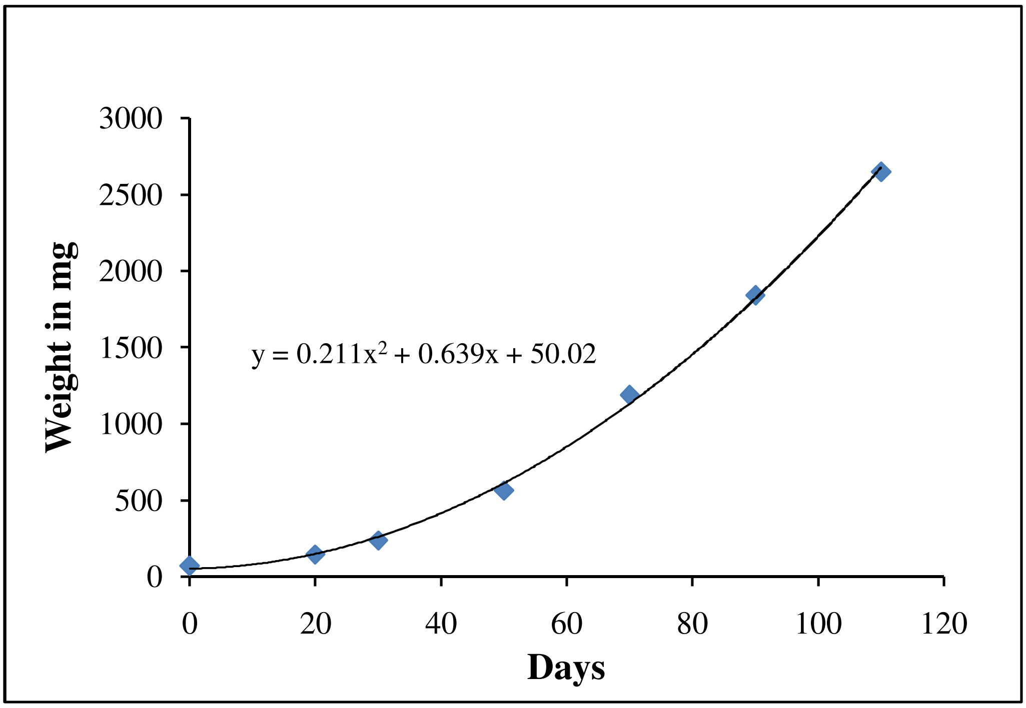

Fig. 7.2. Growth profile of *S. biaculeatus*juveniles in terms of weight duringrearing period.

7.3. Discussion

Captive rearing of *S. biaculeatus* adults

In the present study, the maintenance of *S. biaculeatus* adults and their reproductive performance in captivity is discussed. As of now detailed literature is available on captive breeding and rearing of seahorse species (Vincent, 1990; Mi, 1992; Masonjones and Lewis, 1996; Mi *et al*., 1998; Hilomen-Garcia *et al*., 2003; Silveira, 2000; Woods, 2000; Naik *et al*., 2002; Job *et al*., 2002; Ortega-Salas and Reyes-Bustamante, 2006; Murugan *et al*., 2009; Pawar, 2014). The information pertaining to captive rearing and breeding of pipefish species is limited (Kornienko, 2001; Silva *et al*., 2006a, b; Dhanya, 2008; Barrows *et al*., 2009; Gurkan *et al*., 2009).The knowhow generated during the present study on captive rearing of *S. biaculeatus* would strengthen our understanding towards better culture practices and artificial propagation for better conservation measures.

Courtship behavior and mating

In syngnathids, the role of daily greetings and courtship behaviour is now well understood in respect to their captive breeding (Vincent 1995a, b). In the case of captive black stripped pipefish, *Syngnathus abaster*, Silva *et al*. (2006a) reported that the female initiated courtship followed by brightening, flickering, crossing, parallel swimming and mating rise, and finally spawning. Monteiro *et al*. (2001) also described detailed courtship of *Nerophis lumbriciformis* and reported that females first approached males for selection and courtship. Unlike the swimming and vertical movements in the courtship behaviour of the other pipefish species, this is completely absent in courtship behavior of *N. lumbriciformis*, which suggests its adaptation to intertidal low water level conditions. On the other hand, Kornienko (2001) stated that males of *S. acusimilis* initiated courtship rituals in captive condition and the following steps were quite similar. In the present study, it has been observed that *S. biaculeatus* females initiated the courtship rituals and mating, which is quite similar to other pipefish.

Fecundity and Gestation period

During the present work, fecundity of *S. biaculeatus* was observed to be 155 ± 44 (64 to 215). Number of eggs in *M. crinigerus* was found to vary between 2 and 50 (Powell and Strawn, 1963). Gasparini and Teixeira (1999) observed that the number of hydrated oocytes varied from 36 to 165 in *S. scovelli* in Brazilian coastal waters, while, egg numbers in *S. folletti* and *S. acusimilis* ranged from 30 to 219 and 50 to 500, respectively (Teixeira and Vieira, 1995; Drozdov *et al*., 1997). In *N. lumbriciformes*, the fecundity varied between 23 and 122 (Lyons and Dunne, 2005). In *S. acus*, average hydrated oocyte number in females was 29 ± 4 and average egg number in pouch of pregnant males was 24 ± 5, possibly few eggs might have had droped during transfer (Gurkan *et al.*, 2009). In the case of belly-barred pipefish, *Hippichthys spicifer* in Okinawa-Jima River, Japan, number of eggs in pouch varied from 114 to 1009 with mean of 556 ± 218 (Ishihara and Tachihara, 2009). Takahashi *et al.* (2003) observed 155 matured eggs in the ovary of *S. biaculeatus,* while Dhanya (2008) observed this number to vary between 108 and 236 (153.4 ± 6) in wild caught *S. biaculeatus*. These values are comparable to the mean fecundity (155 ± 44) observed in the present study. On the other hand, higher fecundity (238 ± 57; maximum 351) in *S. biaculeatus* from wild caught males in Papua New Guinea was observed by Barrows *et al*., (2009).

Average gestation period for *S. biaculeatus* was observed to be 18 ± 2 days during the present study at 27 ± 1.5 ºC. Most of the other studies on life history of pipefish species were carried out from wild caught pregnant males, and therefore, the exact data on gestation period in captivity is limited. Dhanya *et al*. (2005) reported that gestation period of *S. biaculeatus* at temperature of 28-32 ºC was 25 ± 5 days. Silva *et al.* (2006a) reported that gestation period of black stripped pipefish, *S. abaster* varied from 24-32 days at 18-19 °C and 21 days 21-22 °C. In the case of Broad nose pipefish, *S. typhle*, gestation period of 17 to 39 days with an average of 25 ± 1 was reported (Mobley *et al*., 2011). Observations made in present study were found to be similar with earlier studies on pipefish species.

Spawning

During captive breeding of *S. biaculeatus*, batch spawning behaviour was observed. Similar observation was made by Silva *et al*. (2006a, b), wherein *Syngathus abaster* juveniles hatched within 2-3 days. In the pipefish, *S. scovelli* the hatching of juveniles from the brood pouch of males occurred at an interval of 24 hours (Azzarello, 1991).

Inter-mating duration

The average time period (in days) between two successive matings of *S. biaculeatus* was observed to be 21 ± 2 days.Though literature (Watanbe *et al*., 1997; Wilson *et al*., 2003; Silva *et al*., 2006a, b) on breeding aspects of pipefishes is available, detailed information pertaining to inter-mating duration of pipefish species is lacking. On the other hand, studies on inter-matingduration of seahorse species are well documented (Vincent, 1995a, b; Kvarnemo *et al*., 2000; Perante *et al*., 2002; Pawar, 2014).

Successful mating and spawning of *S. biaculeatus* was observed 14 times during 12 months during the present study. Franzoi *et al*. (1993) reported that wild caught pipefish species, *Syngnathus abaster* and *S. taenionotus* mated four times and twice respectively in a single breeding season. Silva *et al*. (2006a) observed that three out of eight pairs of *S. abaster* spawned for three consecutives times in an aquarium. The observations made in the present study are similar to the previous ones, suggesting that multiple breeding of *S. biaculeatus* in captivity is achievable.

During breeding trials of *S. biaculeatus* in captivity, it was observed that all individuals mated with their respective partners suggesting monogamous mating pattern under captive condition. Monogamous mating pattern in *S. biaculeatus* was previously reported by few workers from studies on wild caught pregnant males (Takahashi *et al*., 2003; Dhanya *et al*., 2005; Dhanya, 2008; Barrows *et al*., 2009). This type of mating system is reported only in few pipefish species namely *Corythoichthys intestinalis*, *Hippichthys penicillus* and *C. haematopterus* (Gronell, 1984; Watanabe *et al*., 1997; Matsumoto and Yanagisawa, 2001). Polygamous mating has been reported in *N. ophidion*, *S. acusimilis*, *S. floridae*, *S. schlegeli*, *S. scovelli* and *S. typhle* (Jones and Avise, 2001; Kornienko, 2001; McCoy *et al*., 2001; Watanabe and Watanabe, 2002). In the case of *S. typhle* and *N. ophidion*, females are the predominant competitors for mates and showed polygamous mating behaviour (Berglund *et al.*, 1989).

External gas bubble disease (EGBD)

Published literature over EGBD in syngnathid species is not available except Belli *et al*. (2006). Acetazolamide (trade name 'Diamox®') can be used to treat EGBD. Diamox® is a non-bacteriostatic sulphonamide. Acetazolamide inhibits production of the zinc containing enzyme carbonic anhydrase. Its application in treating GBD was pioneered by Dr. Martin Greenwell from Shedd aquarium in Chicago and Dr. Andy Stamper of the Living Seas, USA (Belli *et al.*, 2006). During the present study, this disease was successfully treated by administration of inhibitors of carbonic anhydrase *ie*. Acetazolamide tablet.

Captive rearing of *S. biaculeatus* juveniles

The application of aquaculture technology through responsible re-stocking, stock enhancement and sea ranching programs are being employed in various countries to increase production of capture based fisheries (Tlusty, 2002). Till date, no commercial aquaculture venture of pipefish species exists. In the present work, the captive rearing trials of *S. biaculeatus* juveniles with different live food organisms were tested for better growth and survival.

Early juvenile rearing is one of the challenges in any marine fish mariculture system, and the same is applicable to syngnathid fishes. The rearing of pelagic phase juveniles is most widely faced problem during culture of syngnathid fishes (Takahashi *et al.*, 2003; Dhanya, 2008; Koldewey and Martin-Smith, 2010; Murugan *et al.*, 2009; Pawar *et al.*, 2011). In the present study, pipefish juveniles fed with zooplankton (copepod dominated) exhibited higher growth (228.20 ± 1.62% length gain; 135.40 ± 1.08% weight gain and 476.33 ± 0.3% SGR) and survival rates (100%) as compared to those fed with *Artemia* nauplii. Similar observations were reported in the case of *S. biaculeatus* and Spotted pipefish, *Stigmatopora argus* by Dhanya (2008) and Payne *et al*. (1998), respectively, where copepods resulted in higher growth and survival. It is now well known fact that zooplankton (especially copepods) are naturally rich in polyunsaturated fatty acids (PUFA) particularly eicosapentaenoic acid (EPA; 20:5 *n*–3) and docosahexaenoic acid (DHA; 22:6 *n*–3), and many authors have reported that marine fish larvae required first feeding prey containing high PUFAs for better growth (Payne *et al*., 1998; Meeren *et al*., 2008). On the other hand, *Artemia* species lack these PUFAs naturally, mostly *n* –3 and therefore do not provide adequate nutrition for marine fish larvae (Tlusty, 2002). Feeding *S. biaculeatus* juveniles with zooplankton may provide better nutrition than *Artemia,* which resulted in higher growth and survival during culture period.

In the second experiment, *S. biaculeatus* juveniles were fed with amphipods, mysids and adult *Artemia* for 90 days. The natural diet of pipefish species consists of amphipods, large copepods, decapods larvae, isopods, fish larvae and other zooplankton (Franzoi *et al*., 1993; Teixeira and Musick, 1995; Do *et al*., 1998; Woods, 2002). During captive rearing of *S. biaculeatus* for 90 days, juveniles were fed with amphipods, *Grandidierella* sp. resulted in higher growth in terms of length gain (167.67 ± 0.14%), weight gain (1742.76 ± 1.60%), SGR (782.93 ± 0.03%) and survival (93.33%) when compared to those fed with mysids and adult *Artemia*. In pipefish sub-adult and brood stock feeding, many researchers used amphipods and observed higher growth rate and better breeding performance (Ryer and Boehlert, 1983; Murugan *et al*., 2013; Pawar, 2014). Many mysid species are also proved to be nutritional supplement for marine fishes (Woods and Valentino, 2003; Lipton and Thangaraj, 2006; Olivotto *et al*., 2008; Eusebio *et al*., 2010). In the present study, amphipods and mysids resulted in almost similar growth performance while adult *Artemia* resulted in less growth and survival. In aquaculture, *Artemia* sp. is most commonly used for feeding of fish larvae. However, the cost of *Artemia* is unaffordable to resource-poor farmers in the developing nations, which has necessitated investigation into alternative feed (Olurin and Oluwo, 2010). Mass scale laboratory culture as well as wild collection of mysids has limitations in providing the required need of the hatchery. On the other hand, amphipods, *Grandidierella* sp. can be mass cultured under laboratory conditions (personal observation) and suffice the nutritional requirement of the *S. biaculeatus* juveniles.

Over a 110 days rearing period of *S. biaculeatus* juveniles, total length (TL) of 169.61 ± 2.41 mm and wet weight of 2646.60 ± 165.45 mg was achieved. In juveniles rearing of the same species, Dhanya (2008) reported TL of 194.17 mm and wet weight of 3700 mg over a period of 150 days. The TL attained by *S. biaculeatus* juveniles was higher compared to *Stigmatopora argus* (128 mm after 180 days) showing that growth rate was higher in *S. biaculeatus* (Payne *et al*., 1998). According to Barrows *et al*. (2009), sexual dimorphism in *S. biaculeatus*is easily possible after length of 155 mm.

Highest feeding rates of *S. biaculeatus* juveniles were observed during morning and evening hours as compared to noon, even after provision of illumination. Results of the present study are in agreement with Dhanya (2008). Syngnathid fishes are visual and continuous predators (Foster and Vincent, 2004), therefore feeds were provided in *ad libitum* manner during rearing of juveniles. The observation made in the present study revealed that zooplankton and amphipods form a better source of nutrition as evident by higher growth rates as compared to mysids and *Artemia*.

Chapter 8

Summary

The present study provides comprehensive information on the eco-biological aspects, captive rearing and breeding of alligator pipefish, *Syngnathoides biaculeatus*.

1. The observations made in the present study reveal that the occurrence of *S. biaculeatus* is dependent on the presence of its preferred seagrass habitat. *S. biaculeatus* is more abundant in Palk bay and Gulf of Mannar (south east coast). Patchy distribution has been observed at Neendakkara and Sakthikulangara (South west coast), Lakhshadweep Islands and Andaman Islands along the Indian coast. A new distributional record of *S. biaculeatus* along the coastal waters of Goa suggest bio-geographic significance indicating a possible means of range extension of its native zoo-geographical range.

2. A total of 20 morphometric and 5 meristic counts were studied during the present study. The estimate for the parameter '*b*' of the LWR ($W=aTL^b$) for *S. biaculeatus* was determined to be 1.75 indicating a negative allometric growth pattern (b < 3).Biometric characters, LLRs and LWRs developed for *S. biaculeatus* provide baseline information from this region to enable comparison of the biometry of this species with regard to their occurrence and habitat specificity. Biometric parameters established during the present study would enable to assess species status from different populations around the world.

3. The diet analysis of *S. biaculeatus* suggested that its natural diet comprised micro-crustaceans (~ 90%), which is similar to other syngnathid fishes. The major food items of *S. biaculeatus* were amphipods, copepods, isopods, peracarids, decapods, while sand particles, foraminifera and algal pieces were also observed in their gut contents. This information would envisage developing strategies for artificial rearing and breeding through development of appropriate diets.

4. The results of the present study on proximate composition suggest that the fatty acid and amino acid profile, trace element concentrations as well as C:N ratio of the *S. biaculeatus* were similar to seahorses indicating that this species could be more efficiently used for TCM as well as for human nutrition. Further, the methanolic extract of *S. biaculeatus* showed high total phenolic content and

scavenging potential of free radicals such as DPPH, hydroxyl and reducing the ferric to ferrous ions.

5. The present observations on the reproductive behaviour of *S. biaculeatus* under captive conditions are useful for further advancement in captive breeding of *S. biaculeatus*. In captive rearing studies, zooplankton dominated with copepods and amphipods were found to be the most suitable live food organisms for higher growth and survival. Rearing of *S. biaculeatus* juveniles under captive environment showed potential for development of pipefish commercial cultures. The present investigation showed that alligator pipefish juveniles could be reared in captivity without compromising its growth and health.

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Appendix

Research articles published

1) Sanaye S.V., Rivonker C.U., Ansari Z.A., Sreepada R.A. (2016) A new distributional record of alligator pipefish, *Syngnathoides biaculeatus* (Bloch, 1785) from the coastal waters of Goa, central west coast of India. *Indian Journal of Geo-Marine Sciences*, **45**: 1299-1304.
2) Sanaye S.V., Pawar A.P., Rivonker C.U., Sreepada R.A., Ansari Z.A., Ram A. (2016) Biochemical composition of alligator pipefish, *Syngnathoides biaculeatus*. *Chinese Journal of Oceanology and Limnology*, DOI: http://dx.doi.org/10.1007/s00343-017-6070-0.

Research articles accepted

1) Sanaye S.V., Rivonker C.U., Sreepada R.A., Ansari Z.A. Natural diet of the alligator pipefish, *S. biaculeatus* inhabiting Palk Bay, southeast coast of India. Journal name - Indian Journal of Geo-Marine Sciences

2) Sanaye S.V., Rivonker C.U., Sreepada R.A., Ansari Z.A., Murugan A., Ramkumar B. Weight–length relationship and Fulton's condition factor of the alligator pipefish, *Syngnathoides biaculeatus* (Bloch, 1785) from southeast coast of India.

Journal name - Current Science