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Marine Fisheries Information Service Technical & Extension Series

The Marine Fisheries Information Service, Technical & Extension Series (MFIS) is a quarterly publication of ICAR-Central Marine Fisheries Research Institute disseminating latest research information on marine fisheries and mariculture in India. Research based technical articles, reporting significant new information, knowledge and understanding of marine fisheries and ecosystems as well as new concepts/technologies in marine fish nutrition, hatchery and larval rearing, fish pathology, fish health management, application of genetics in fish conservation and farming, sea farming technologies, seafood trade and fisheries governance are published. To see all issues since 1978, visit:

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Sorted sciaenid catches at New Ferry Wharf, Mumbai

Photo credit: Akhilesh, K.V.

Marine Fisheries Information Service Technical and Extension Series envisages dissemination of information on marine fishery resources based on research results to the planners, industry and fish farmers and transfer of technology from laboratory to the field.

MFIS

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From the Editorial Board

Warm greetings to all our esteemed readers

In the year 2020, the global fisheries and aquaculture production reached a record production of 214 million t which was worth USD 424 billion and provided food, livelihood and economic benefits to several million people (The State of World Fisheries and Aquaculture, FAO, 2022). The seafood trade was valued at US$151 billion and nearly 60% of the volumes traded reportedly originated from developing countries. The report further mentions although the global trend of increasing number of overfished stocks is showing signs of reverse, issues of overfishing, pollution, poor fisheries management and climate change remain major challenges. Focus on certification for sustainable fishing, ecolabeling and blockchain technology enabled traceability in seafood value chains are emerging globally. The aim is to ensure resources are exploited optimally through appropriate fishing controls. Hence, science-backed, effective fisheries management policies will be of crucial importance in the coming years. In this issue of MFIS, we introduce a section "Science Corner". It briefly communicates salient research findings of contemporary interest in the marine fisheries sector, published in peer-reviewed journals.

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Contents

Research Communications

Diversity and present status of croaker fishery along Odisha Coast

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Abstract

Croakers are locally called as "Patharamundi", "Sila" or "Borei" are commercially important demersal resources exploited all along the Odisha coast. The diversity and status of croaker fishery along Odisha coast in the Bay of Bengal is reported. Landings of croakers showed an increasing trend over the decades with lowest catch recorded in 1976 (333 tonnes) and peaked in 2011 (46,468 tonnes) after which the catch has been declining till 2018. Among the maritime states of India, Odisha, contributing 20,625 tonnes accounting for 18% of the country's total croaker landings in 2020, showed a tremendous increase compared to the previous year. It was a significant component in the trawl catches forming 34% of the total demersal fish landings and 12% of the total marine fish landings of Odisha. Lesser sciaenids (49%) dominated the croaker landings. They were caught by various gears such as trawl nets, gillnets, hook & line, ringseines, and shoreseines. Bulk of the catches were taken by trawl nets (73%) followed by gillnets (22%). More than 20 species contribute to the fishery of which the major species were "lesser sciaenids" such as *Johnius carutta* (40%), *Pennahia anea* (30%), *Panna heterolepis* (12%), *Kathala axillaris* (9%), *Nibea maculata* (5%). *Otolithes ruber* (66%), *Chrysochir aureus* (23%), *Pterotolithus maculatus* (7%) dominated in the "intermediate sciaenids" and *Otolithoides pama* among the "greater sciaenids" with 98% contribution. *Protonibea diacanthus* locally called as "Telia" is gaining attention among the fishermen in the recent past due to their high quality swim bladders which are used for the production of isinglass for export. As compared to previous years, the lowest catch was reported in 2020 (0.30 tonnes) probably due to non-fishery causes such as Covid pandemic which affected general fishing activities.However, continuous monitoring and management of this economically important species is required. Juveniles and young adults of several species of croakers in bottom trawls as bycatch can cause an economic loss to the fishery sector and require management measures.

***Keywords**: Bay of Bengal, Odisha, diversity, fishery, croaker, Sciaenidae*

Introduction

The fishes of the Family Sciaenidae commonly called as drums or croakers, contributed by small to large high-quality food fishes. The air bladder of some species (e.g. *Protonibea diacanthus*) are used to produce isinglass for industrial uses and an esteemed Food item in some culture. In 2020, croakers constituted about 4.3% of India's total marine fish production with northwestern region contributing nearly 37% followed by northeastern region 36% to the total croaker landings. Among the maritime states, Gujarat landings was 31,411 t (27%) followed by West Bengal 22,114 t (19%), Odisha 20,625 t (18%) and Maharashtra with 12,108 t (10%). The present study documents the diversity and status of croaker fishery along Odisha coast based on observation recorded from Ganjam, Puri, Jagathsingpur, Kendrapara, Bhadrak and Baleshwar fishery centres.

Catch trend

The landing of croakers registered an increasing trend over the decades along the Odisha coast during 1976-2020 with lowest catch of 333 t recorded in 1976 and peak 46,468 t in 2011 after which the landings show a decline till 2018 (Fig. 1). During 2020, croakers stood first position with 20,625 tonnes accounting for 34% of the total demersal fish landings of the state. Group-wise analysis revealed that "lesser sciaenids" constitute 10,076 tonnes of the state total croaker landings followed by "intermediate sciaenids" (8,623 tonnes) and lowest by "greater sciaenids" (1,926 t) (Fig. 2). The highest landings occured during July-September with 8,670 t (42%) followed by 7,881 t in October-December (38%), 3,732 t in January-March (18%) and lowest of 341 t in April-June (2%) corresponding with the monsoon fishing ban period (Fig. 3). Of the total croakers landed, 18,657 t were caught by mechanized fishing vessels, 1,117 tonnes by motorized and 851 tonnes by non-mechanized fishing

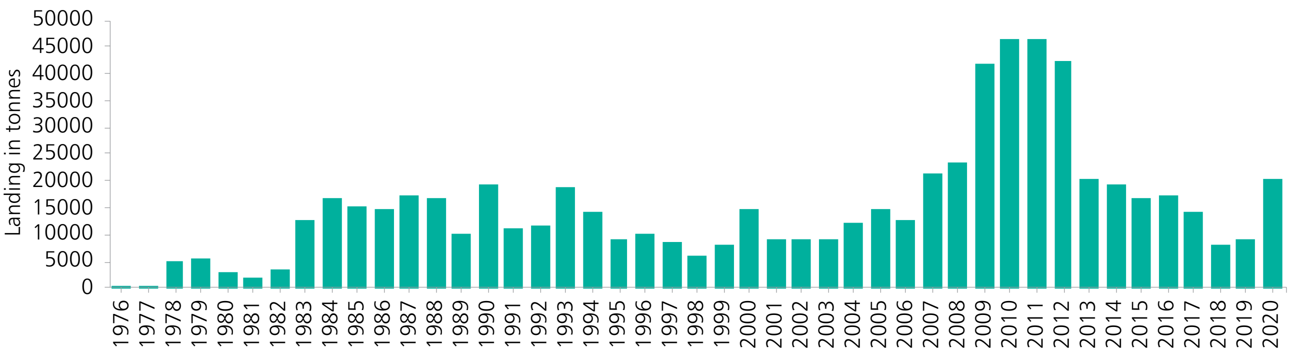


Fig. 1. Catch trend of croakers along Odisha coast during 1976-2020

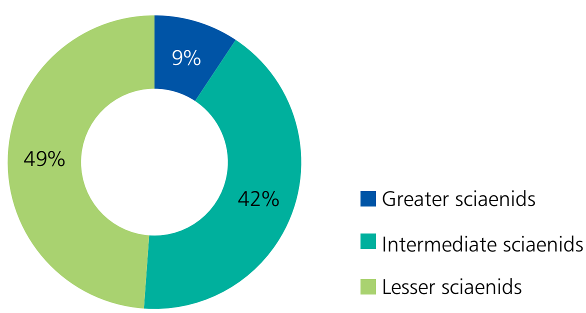


Fig. 2. Group-wise contribution of croaker landings along Odisha coast in 2020

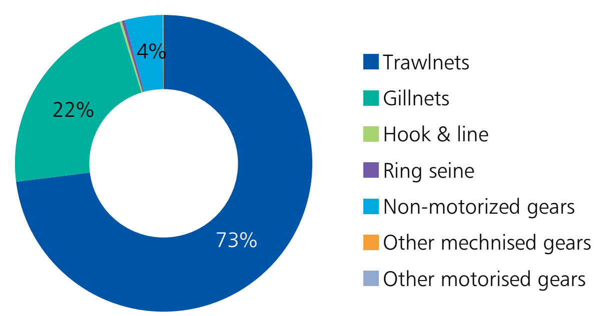


Fig. 4. Gear-wise contribution to the total croaker landings of Odisha coast during 2020

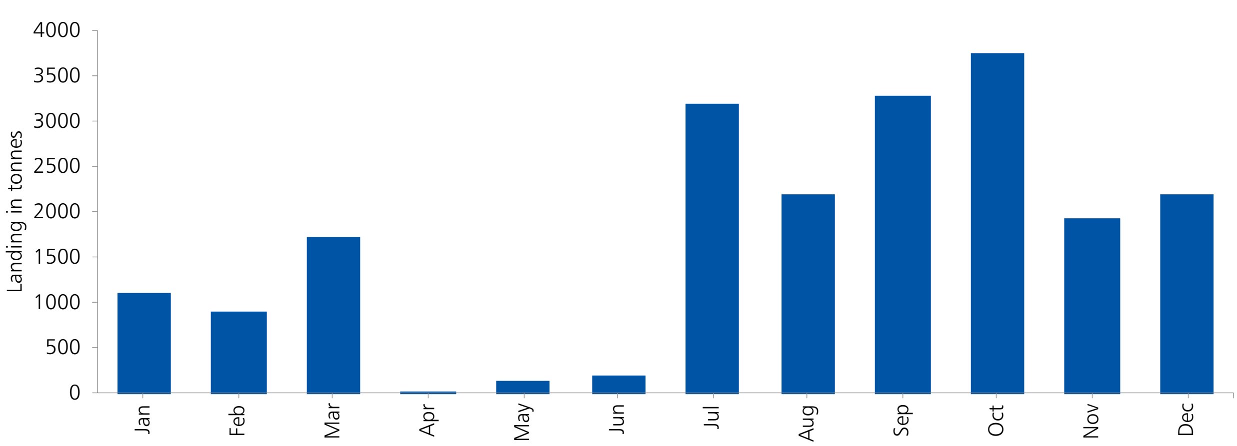


Fig. 3. Month-wise landing of croakers along Odisha coast in 2020

crafts. Among the various fishing gears operated along the coast trawlnets contributed to the highest catch followed by gillnets (Fig. 4).

Species composition

In the present study, the croaker species were categorized based on the maximum reported size as greater sciaenids (> 100 cm), intermediate sciaenids (50-100 cm) and lesser sciaenids (< 50 cm). More than 20 species of croakers in 14 genera were recorded during the study period 2017-2020 (Table 1). Major species contributing to the croaker

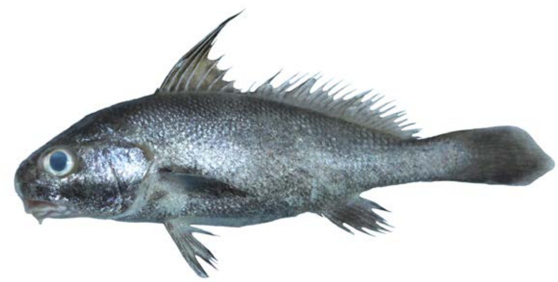
Table 1. List of croaker species recorded along Odisha coast during 2017-2020

Species	Common name	Local name	Length range observed (TL mm)	Gears caught	Frequency of occurrence in landings
Protonibea diacanthus (Lacepède, 1802)	Blackspotted croaker	Telia	600-1260	Trawlnet, hook & line, gillnet	Occasional
Otolithoides biauritus (Cantor, 1849)	Bronze croaker	Badia sila, Jamuna borei	420-1140	Trawlnet, hook & line, gillnet	Occasional
Otolithoides pama (Hamilton, 1822)	Pama croaker	Mohana sila, Jamuna borei	130-418	Gillnet, trawlnet, shoreseine	Common
Macrospinosa cuja (Hamilton, 1822)	-	Paru sila	330-600	Gillnet, trawlnet	Rare
Otolithes ruber (Bloch & Schneider, 1801)	Tigertooth croaker	Silver sila, Dantura	157-490	Gillnet, trawlnet, hook & line, ringseine	Common
Pterotolithus maculatus (Cuvier, 1830)	Blotched tiger-toothed croaker	Biradia sila, Baghada borei	160-500	Gillnet, trawlnet, shoreseine	Common
Chrysochir aureus (Richardson, 1846)	Reeve's croaker	Musura sila, Musura	132-440	Gillnet, trawlnet, hook & line, shoreseine	Common
Daysciaena albida (Cuvier, 1830)	Bengal corvina	Parua sila, Telia borei	400-691	Gillnet	Rare
Nibea soldado (Lacepède, 1802)	Soldier croaker	Telia borei	185-257	Gillnet, trawlnet	Common
Nibea maculata (Bloch & Schneider, 1801)	Blotched croaker	Gadi sila /tiger sila/Alata pati borei	118-274	Gillnet, trawlnet	Common
Atrobucca trewavasae Talwar & Sathirajan, 1975	-	Meigalia sila/Tetra borei	200-370	Trawlnet, gillnet	Occasional
Panna heterolepis Trewavas, 1977	Hooghly croaker	Gadra sila, /nagudia sila/ Jamuna borei	150-210	Gillnet, trawlnet, shoreseine	Common
Pennahia anea (Bloch, 1793)	Donkey croaker	Thikiri sila, Borei, Patharamundi	106-210	Gillnet, trawlnet, shoreseine	Common
Pennahia ovata Sasaki, 1996	-	Thikiri sila, Borei, Patharamundi	100-414	Gillnet, trawlnet, shoreseine	Common
Kathala axillaris (Cuvier, 1830)	Kathala croaker	Mundi sila, Borei, Patharamundi	133-166	Gillnet, trawlnet, ringseine	Common
Johnius amblycephalus (Bleeker, 1855)	Bearded croaker	Dadhi borei, Thikiri sila, Chocolate borei	71-220	Gillnet, trawlnet	Occasional
Johnius belangerii (Cuvier, 1830)	Belanger's croaker	Thikiri sila, Borei,	80-240	Gillnet, trawlnet	Occasional
Johnius borneensis (Bleeker, 1851)	Sharpnose hammer croaker	Thikiri sila, Borei, Patharamundi	185-252	Gillnet, trawlnet	Common
Johnius carouna (Cuvier, 1830)	Caroun croaker	Thikiri sila, Borei, Patharamundi	90-220	Gillnet, trawlnet	Occasional
Johnius carutta Bloch, 1793	Karut croaker	Sila, Borei, Patharamundi/ Tetra borei	125-220	Gillnet, trawlnet, ringseine	Common
Johnius dussumieri (Cuvier, 1830)	Sin croaker	Thikiri sila, Borei, Patharamundi	75-183	Gillnet, ringseine, trawlnet, shoreseine	Common
Johnius macropterus (Bleeker, 1853)	Largefin croaker	Thikiri sila, Borei, Patharamundi	100-150	Gillnet, trawlnet	Occasional
Johnius macrorhynus (Lal Mohan, 1976)	Big-snout croaker	Thikiri sila, Borei, Patharamundi	126-240	Gillnet, trawlnet	Common
Dendrophysa russelii (Cuvier, 1829)	Goatee croaker	Thikiri sila, Borei, Patharamundi, Dadhi borei	160-195	Gillnet	Occasional

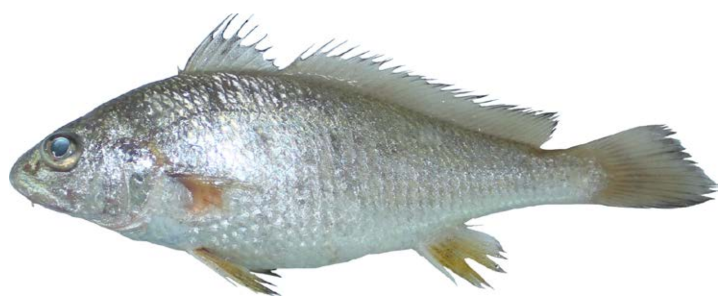
Pennahia anea 162 mm TL

Kathala axillaris 151mm TL

Panna heterolepis 150 mm TL



Johnius amblycephalus 97 mm TL



Johnius macropterus 126 mm TL

Johnius carutta 189 mm TL

Johnius borneensis 210 mm TL

Johnius macrorhynus 213 mm TL

Johnius dussumieri 137 mm TL

Fig. 5. Major species of lesser sciaenids recorded along Odisha coast during 2017-2020

Pterotolithus maculatus 488 mm TL

Otolithes ruber 370 mm TL

Chrysochir aureus 225 mm TL

Daysciaena albida 509 mm TL

Fig. 6. Major species of intermediate sciaenids recorded along Odisha coast during 2017-2020

Protonibea diacanthus 843 mm TL

Otolithoides biauritus 398 mm TL

Macrospinosa cuja 320 mm TL

Otolithoides pama 418 mm TL

Fig. 7. Major species of greater sciaenids recorded along Odisha coast during 2017-2020

fishery were *Otolithes ruber* (28%) followed by *Johnius carutta* (20%), *Pennahia anea* (15%), *Chrysochir aureus* (10%), and *Otolithoides pama* (9%). Lesser sciaenids are the most abundant and dominated the croaker fishery (Fig. 5) followed by intermediate (Fig. 6) and greater sciaenids (Fig. 7). The major species contributing to the lesser sciaenid fishery were *J. carutta*, *P. anea*, *Panna heterolepis*, *Kathala axillaris* and *Nibea maculata* (Fig. 8). Similarly, *O. ruber*, *C. aureus* and *Pterotolithus maculatus* were the major species contributing significantly to the intermediate sciaenid (Fig. 9). Among the greater sciaenids *O. pama* contribute about 98% of the catch whereas

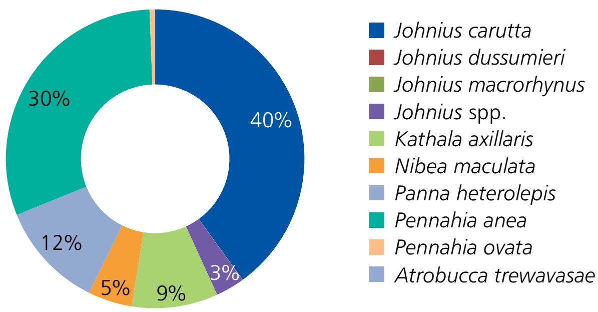


Fig. 8. Species-wise contribution to lesser sciaenid landings along Odisha coast during 2020

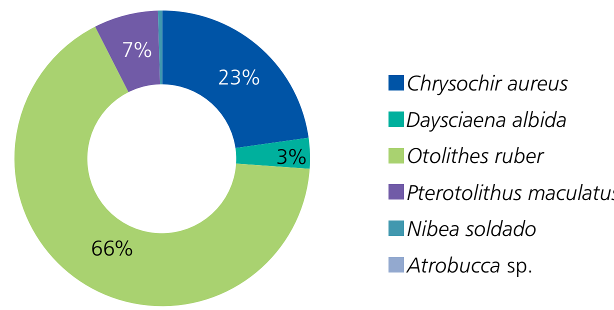


Fig. 9. Species-wise contribution to intermediate sciaenid landings along Odisha coast during 2020

Table 2. Some landing records of *Protonibea diacanthus* along Odisha coast during 2017-2020

Date of landing	Place of landing	Gear used	Depth of fishing (m)	Numbers caught	Total length (cm)	Weight (kg)	Price per kg (₹)
08.03.2018	Pentakota	Drift gillnet	30-40	1	120	26.0	3000
23.03.2019	Paradeep	Trawl net	55	1	105	19.0	7000
28.03.2019	Paradeep	Trawl net	50	1	75	4.0	1000
16.07.2019	Atharbanki	Long line	50	3	60-120	3.5-28.0	1000-6300
17.08.2019	Balaramgadi	Trawl net	60	1	65	3.8	1875
17.09.2019	Atharbanki	Long line	60	7	60-126	3.5-31.5	1100-7350
06.11.2019	Atharbanki	Long line	55	3	112-122	24-30.0	6000-7000
18.11.2019	Atharbanki	Long line	50	2	115-120	27-29.5	5500-6800
19.11.2019	Atharbanki	Bottom set gillnet	35 z	1	108	23.0	5500
4.12.2019	Atharbanki	Long line	35	1	98	18.0	4500
17.12.2019	Atharbanki	Bottom set gillnet	35	1	75	4.0	1000
13.01.2020	Paradeep	Long line	45	1	122	28.5	6200
28.01.2020	Paradeep	Long line	50	1	104	19.0	4000

O. biauritus, *Protonibea diacanthus* occur occasionally and *Macrospinosa cuja* very rarely.

Protonibea diacanthus, locally known as "Telia" is one of the most commercially important croaker species and is gaining more attention among the fishermen. The excellent quality of swim bladders of these species fetches very high price in the international markets. Fishes with swim bladders are usually sold at ₹1000-7350 per kg body weight at landing centres depending upon the sizes (Table 2). Airbladders are dissected out, dried and sold separately at ₹40,000-50,000 per kg in the local markets or sent to nearby markets such as Digha or Calcutta for getting better prices. Male fish air bladders are considered superior in quality compare to those from female fishes. After removing the air bladder, fishes are filleted and processed both for local (₹400-600 per kg) and export markets. Recently, Jakhar *et al*. (2012) confirmed that the skin gelatin of this species can be used in food applications to extend the gelatin market to religious groups which do not accept the porcine and bovine gelatin. The estimated landings of this species during the period 2007-2020 indicates a declining trend with highest landings of 1,227 t recorded in 2010, followed by continuous decline. Most of the catches recorded during the present study were represented by large sized fishes with a narrow length range of 60-126 cm TL (Table 2). Most of the species in the greater sciaenid group have a long life span, larger maximum size, late age and size-at maturity, lower spawning frequency, high batch fecundity, and low relative fecundity compared to the other sciaenid (Waggy *et al*., 2006) which makes them highly susceptible to overfishing. Thus, capturing mostly large sized fishes will affect the spawning stock

Fig. 10. *Protonibea diacanthus* (120 cm TL & 26 kg TW) landed at Pentokota, Puri, Odisha

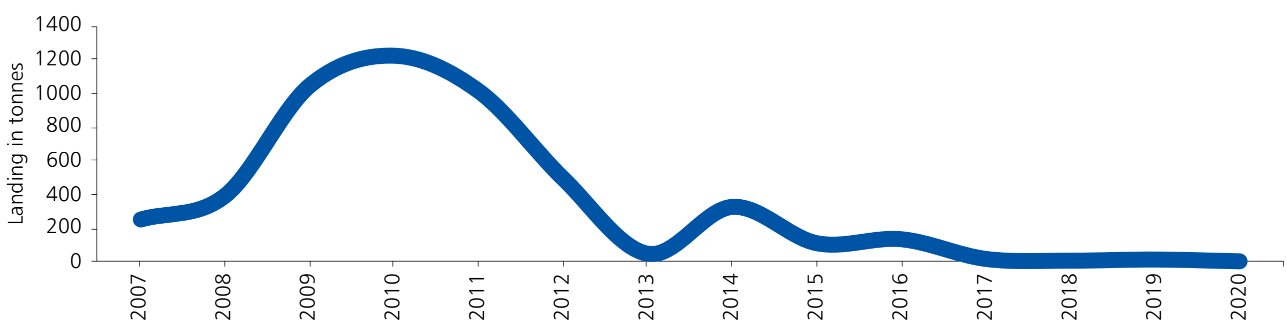


Fig. 11. Catch trend of *Protonibea diacanthus* along Odisha coast during 2007-2020

biomass through recruitment overfishing. Several studies have indicated that the species forms aggregations as a result of seasonal migrations in response to availability of food or based on the species' reproductive cycle and hence capture trends can impact the resource. Hence, continuous monitoring of such economically important species is essential to ensure long term sustainability.

Market trends

The dried air bladders called fish maws are exported mainly to China and Singapore for the manufacture of isinglass, which is used in beverage and cosmetic industry as well as Chinese traditional medicines (Dutta *et al*., 2014). The lesser and medium sciaenids are mostly sold in fresh condition at the local markets or iced and transported to distant interior markets of Odisha, West Bengal, Karnataka and Tamil Nadu for fresh consumption. The lesser sciaenids are salted and sundried onboard multiday trawls and brought ashore as dry fish for consumption. Lesser sciaenids (e.g. *Johnius* spp.) and high valued intermediate sciaenids like *O. ruber* and *C. aureus* are also dried when huge quantities of small sized fishes landed during the peak fishing season. The catches of very small juveniles and the lesser sciaenids of poor quality fishes goes for making fish meal

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Potential of laboratory developed marine bacterial consortium as antibacterial and growth promoting agent in mariculture

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Mariculture is the fastest growing subsector of aquaculture in India, and it provides a significant contribution to animal production and is of economic importance. Technology of marine cage culture in India is pioneered by ICAR-Central Marine Fisheries Research Institute for providing an additional livelihood for the upliftment of economic status of coastal communities, and this transfer of technology was successfully implemented throughout coastal India. Indian Pompano, *Trachinotus mookalee* is the most potential species for marine cage farming in India. Management of water quality and fish health in culture systems is a great challenge in mariculture. Emergence of environmental issues and disease occurrence becomes inevitable due to the increasing demand for intensification and commercialization in mariculture production. In order to manage the culture systems for attaining sustainable production, application of probiotics is the best tool, to achieve better growth rate, survival and disease resistance of fish. Marine microorganisms are identified as potential source of enzymes which may enhance host digestion since they have greater capacity of adhesion to gastrointestinal mucus and tissues. There are several reports of research works on the application of probiotics in aquaculture though very few studies relate its usage in finfish culture. Several commercial probiotic bacteria are being used in aquaculture industry, especially in shrimp aquaculture, but the role of its application in marine finfish culture needs to be established. Hence, the present study was undertaken to evaluate the efficacy of marine probiotic consortium (MPC) (developed in laboratory) on survival and growth of *Trachinotus mookalee*.

Experiment on efficacy of laboratory developed probiotic bacteria belonging to genera *Bacillus, Paenibacillus and Shewanella* (isolated from fish gut) on survival and growth of Indian pompano.

Hundred fingerlings, with an initial average weight of 30g, were kept in one tonne tanks. Fishes, designated as control were fed with pelleted feed at 6% fish biomass. In experimental treatments (T1: laboratory developed probiotics and T2; T3: commercial probiotics), the fishes were fed with commercial pelleted feed (6%), supplemented with the probiotics at a concentration of $1x10^6$cfu/g. Triplicates were maintained for each treatment. Growth and survival of the fished were estimated using standard formulas. Water quality parameters were monitored at fortnightly intervals. Microbiological analysis of the fish gut was undertaken at monthly intervals. All statistical analyses were performed using SPSS software.

Experiment on efficacy of probiotics (T1, T2 and T3) on growth and survival of Indian Pompano revealed a significant increase in average weight (g), % weight gain and specific growth rate (% SGR) of fish in T1 and T2, when compared to T3. Final average weight (g), average daily growth rate (ADGR g day^{-1}) and specific growth rate (SGR % day^{-1}) of fish at 90 days of experiment were recorded in T2 and T1 as 180 g and 130 g, 1.67g/day and 1.12g/day, 0.87 and 0.71 respectively. Details on growth parameters of *T. mookalee* supplemented with marine probiotic consortium are given in Table 1.

100% survival of fish was recorded in T1 and T2, followed by 85% in T3 and 75% in control at 90 days of culture (Fig. 1). No significant variation in % survival of fish was found between days of culture ($p>0.05$). A positive correlation ($r=0.89$, 0.94, 0.86 in T1, T2, T3, respectively)

Table 1. Growth parameters of *T. mookalee* supplemented with three different microbial consortia

Parameter	T1	T2	T3	C
Initial Biomass (kg)	3	3	3	3
Final Biomass (kg)	13	18	9.8	6.75
Survival %	100	100	85	75
Weight Gain (g)	100	150	85	60
% weight gain	333.34	500	283.34	200
Average Daily Growth rate (g/day)	1.12	1.67	0.95	0.67
Specific growth rate (%/day)	0.71	0.8646	0.6484	0.53
Initial Biomass (kg)	3	3	3	3
Final Biomass (kg)	13	18	9.8	6.75

was observed between % survival and concentration of probiotic bacteria supplemented in all treatments.

Average weight (g), % weight gain, ADGR (g/day) and SGR (%/day) of Indian pompano at 90 days of culture significantly varied between treatments ($p<0.05$) (Figs. 2, 3, 4 and 5). Increasing trend in weight gain of fish was recorded in T2 and T1, but random variations were

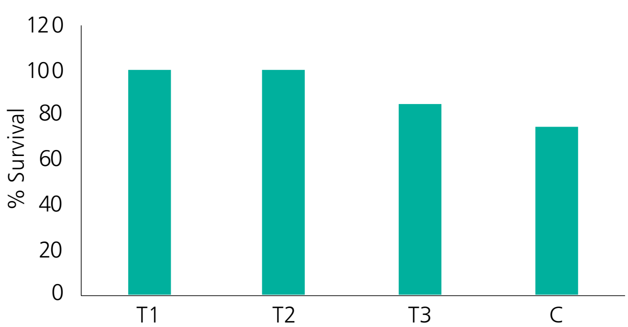

Fig.1 Survival % of Indian pompano with dietary supplementation of MPC

observed in T3 and control, with a decline at 45 days. Weight gain of fish varied significantly ($p<0.05$) between days of culture and treatments.

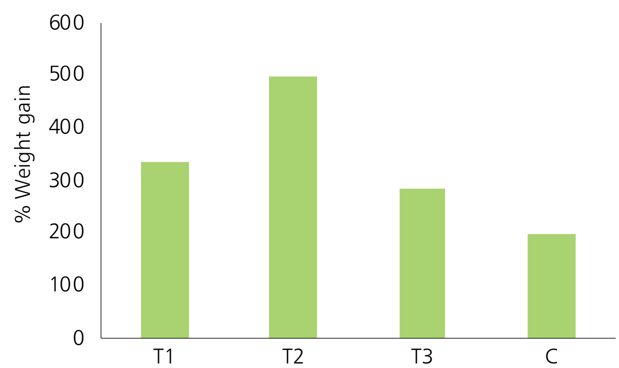

Fig. 3. Weight gain % at different treatment compared to control

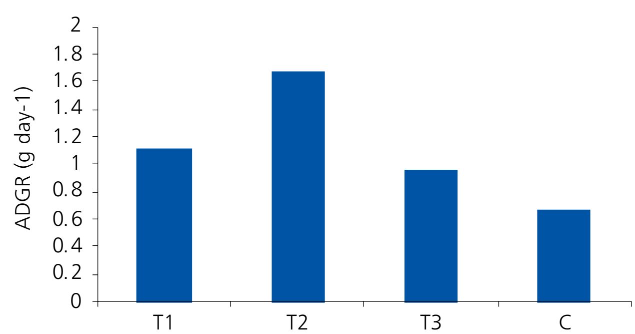

Fig. 4. ADGR of Indian Pompano under different treatments

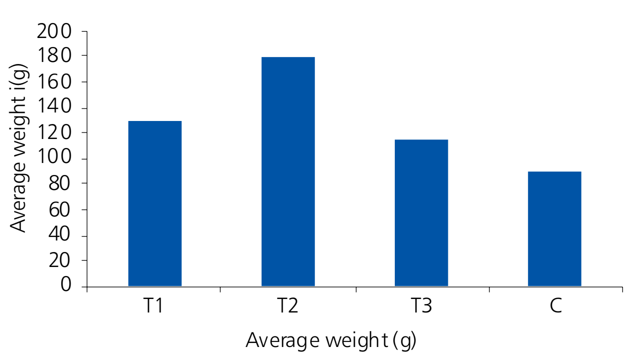

Fig. 2. Average weight (g) of Indian pompano with dietary supplementation of MPC at 90 days of culture

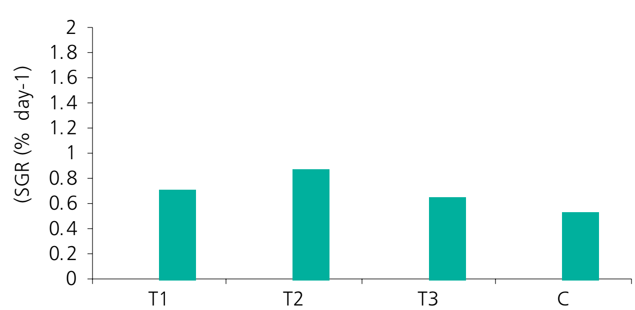

Fig. 5. Specific growth rate (SGR) of Indian Pompano under different treatments

Table 2. Water quality parameters in experimental treatments

	T1	T2	T3	C
Temperature °C	28.5±0.2	28.9±0.3	29.0±0.4	29.1±0.1
Salinity ‰	28±2	28±3	27±3	30±2
Dissolved Oxygen (mg l^{-1})	5.8±0.1	6.1±0.2	4.2±0.3	4.1±0.4
pH	8.1±0.03	8.2±0.03	8.0±0.03	8.3±0.04

Water quality parameters

Temperature (°C), salinity (ppt), dissolved oxygen and pH of water in all the treatments and control are given in Table 2. No significant variation in water quality parameters were observed between the treatments and days of culture ($p>0.05$). Significantly, higher values of ammonia, nitrate and nitrite were recorded in T3 and control ($p<0.05$).

Analysis of gut microbes

Total Viable Heterotrophic bacterial count (TVHC) and Total *Vibrio* loads (presumptive) of water and fish gut were monitored at monthly intervals. TVHC in fish gut varied between 2.8 x 10^{12} to 3.2 x 10^{15} cfu/g in T2 at 90 days of experiment (Fig. 6). A significant variation was recorded between the initial and final Vibrio counts (Presumptive) in all the treatments ($p<0.05$). A significant variation in bacterial diversity between the treatments was recorded. In T2 and T1, *Shewanella* spp., *Paenibacillus* spp. and *Bacillus* spp. were the most dominant bacteria; whereas, in T3, *Bacillus* spp. are the most dominant. A significant increase in *Vibrio* loads of gut was recorded in fishes of control group. However, in T1 and T2, a declining trend in *Vibrio* loads was noted with application of probiotic consortium (r =–0.92).

The findings of the study indicated that the marine probiotic consortium (laboratory developed) was found to be highly potential, and it can be used as feed probiotics for enhancement of growth and production of Indian Pompano. The study showed promising results in enhancement of survival (%), ADGR (g/day) and SGR (%/day) of Indian pompano; which resulted in significant increase of biomass in fish supplemented with MPC. The supplementation of multistrain probiotic played an important role in elimination of *Vibrio*s and in enhancement of growth and survival of *T. mookalee.* In conclusion, the experiment demonstrated supplementation of laboratory developed probiotic consortium (isolated from host gut) in positively impacting growth enhancement and vibriocidal activity. The laboratory developed marine microbial consortium can be recommended as a natural alternative to antibiotics, and as a novel tool for better health management practices in mariculture.

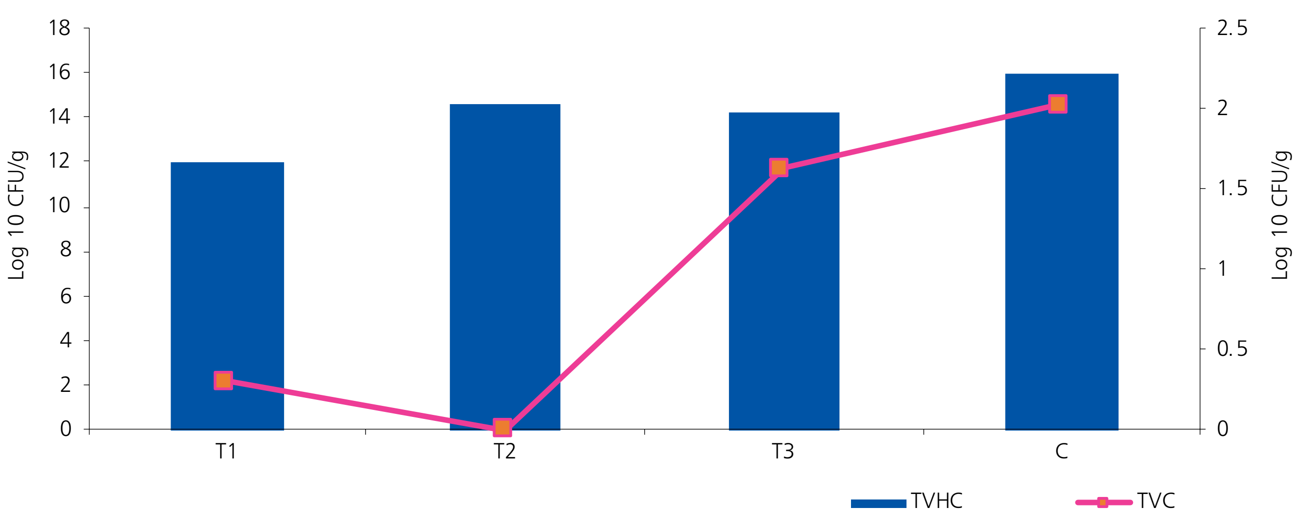

Fig. 6. Total Viable Heterotrophic Bacterial Count and Total Vibrio Count of fish gut

Brief communication

Technology for farming of Orange spotted grouper in marine cage culture systems

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Introduction

Aquaculture of groupers is carried out in tropical and subtropical areas throughout the world, with registered total production of 155 000 t during 2017 (FAO, 2017). Approximately 95% of this production was registered from Asia with major contribution from China (65%), Taiwan Province of China (17%), Indonesia (11%) and Middle East (0.1%). Grouper aquaculture is notable for its high level of diversity, including 47 grouper species and 15 hybrids. Orange spotted grouper, *Epinephelus coioides* valued for its excellent texture and flavour and has good demand in international live reef fish trade, especially in South East Asian countries. Aquaculture of groupers in India using hatchery produced seed was initiated during 2016 – 2017 by Visakhapatnam Regional Centre of ICAR-Central Marine Fisheries Research Institute (CMFRI), following the success in continuous seed production of the species. Initial trials of in sea cages were conducted in different states such as Andhra Pradesh, Tamil Nadu, Kerala, Karnataka and Gujarat. Thereafter, the cage culture technology was demonstrated to different groups including, fishermen, fisheries societies and small entrepreneurs with financial support from the National Fisheries Development Board

Marine fish cage farm at Visakhapatnam

(NFDB), Government of India. Various steps involved in cage culture of the species have been standardised and are explained below.

Cage site selection

As one of the most important factors for cage culture, the selected site should meet the following criteria: Water temperature: 26–30°C, water depth 6-10 m, and away from polluted or industry run offs. Continuous and free water movement, to maintain good dissolved oxygen content of more than 3.5 ppm always which also helps to avoid parasites, is required. Easy accessibility and free from social disturbances should be considered.

Construction

Circular shaped HDPE cages of 6 m diameter inner collar pipe and 8 m diameter outer collar pipe are supported by 8 base supports, 8 vertical supports and 8 diagonal supports. HDPE braided nets are suitable with specification for outer nets of 63 ply, 40 mm mesh size and with net dimensions of 7 m diameter and 4 m depth. Inner HDPE nets are of 63 ply, 25 mm mesh size and with dimensions of 6 m diameter and 4 m depth. The mesh size for inner net varies with the growth of the fishes stocked in the cages, and a mesh size of 10 mm is used till the stocked fish reaches 100g. Bird's net of 80 mm nylon mesh are preferred for fish culture. The cage structure is stabilised in the sea with the help of mooring systems supported by 1.5-2.0 tonne capacity cement blocks/gabion boxes/ anchor systems, with the help of mooring chain (long link alloy steel chain of 13 mm diameter with 22 tonne shearing strength), D-shackles (6.5 tonnes) and swivel (32 mm). Ballast pipes help to maintain the cage structure intact in proper shape against the water movement. To provide sufficient space for fish movement, the inner net has to be tied with two ballast pipes; one at the bottom and the other at the middle of the inner net for avoiding closure of the net due to the water movement.

Nursery rearing

This is an intermediate step after juvenile groupers (2-3 cm) from the hatchery are reared until they reach an optimum size to be stocked in sea cages (20-25g). Generally, this is done in onshore tank facilities established near the grow-out culture for ease of fish transfer. Two types of nursery systems: 1) Indoor system: Flow-through based FRP or concrete tank culture 2) Outdoor culture: Hapa based nursery rearing in earthen ponds or in sea cages are available. Among these, indoor culture system is better as less size variation, higher survival and better ease of operation.

In both nursery systems, feed with high nutrient content (crude protein 45% and crude fat 10%) is suggested, but chopped or minced trash fish is the most preferred by the grouper. In indoor based nursery system, the fingerlings showed good response to both artificial and pelleted feeds, but pellet feed prepared as the use of minced meat deteriorates the water quality quickly. However, in outdoor based nursery culture, a mix of artificial and pelleted feed for efficient feeding is recommended. The feeding rate in nursery is 8 to 7% and 15 to 13% for pelleted feed and trash fish, respectively. The stocked fish fry of 2 to 3 g usually takes 75 days to reach about 20 to 25 g in size. Feeding frequency of 4-5 times per day at 10-7% of body weight is recommended during the initial nursery phase. The commonly available nursery feeds are: Skretting (Norway), Lucky star (Singapore), Uni-President Enterprises Corporation (Taiwan), Growel Feeds Pvt Ltd (India) and Ananda Feed Pvt Ltd (India). Growth rate is slow during the initial growth phase till it reaches 250g, and thereafter increases. Hence, nursery culture of Orange spotted grouper is an important factor in cage culture operation for reducing the culture duration. Due to size variation during nursery rearing cannibalism is observed. To avoid cannibalism, it is suggested to maintain uniform size of the fish by fortnightly grading of the fishes. Survival rate during nursery phase varies from 90-95% in indoor systems and 70-85% for outdoor systems. Proper feeding and fortnight grading helps to improve the survival rate during nursery phase

Nursery rearing of orange spotted grouper fingerlings in hapa in cage

Grow-out culture

Grow out culture is the period following nursery rearing till marketing of the fish. The nursery reared fish seeds are transported to cages either in oxygen filled polythene bags or in containers supported with oxygen where they are slowly released for acclimatising to the cage water environment. The optimum stocking density suggested for the fish is 20 kg of final production per cubic meter area. Therefore, net with 6 m diameter and 4 m depth will occupy an approximately 110 cubic meter area which can produce 2.0 tonnes of fishes. To achieve the estimated production total 2500 seeds should be stocked with an expected survival of 80% and final body weight of around 1.0 kg. Groupers are demersal and always remains at the bottom. Hence net with low depth will help for higher visibility of feed. Therefore, at the beginning of the culture, the net depth of 2.0 m should be preferred. Once the fish size exceeds 250g, then the net depth can be maintained as usual. Artificial floating pelleted feed with high protein is recommended for grow out systems. It requires bigger sized pelleted feed during grow-out since the mouth size is bigger compared to other fishes of same size group. Feed size is most important for efficient feeding. In cages, fish fed with only artificial feed showed good growth response, but size variations were observed. However, feeding with chopped low value fishes not only reduced this size variation but also exhibited good growth. When pellet feeds are applied, feed mesh of 1.0 meter depth should be attached in the inner net to avoid feed wastage. For better feed digestion and assimilation, a minimum time gap of 3 hours should be given between two feeding schedules and the feeding frequency decided accordingly. Feeding is required at least twice a day to maintain good health of the fishes. Fish growth should be monitored fortnightly and feeding rate adjusted based on the weight gain after every sampling. Based on several demonstrations, it is observed that if the fish fingerlings of 20 to 25g are stocked at 20 numbers per m^3, then it takes nearly 12 months for it to reach the size range of 650 to 800 g and approximately 1.5 kg is reached in 15 to 16 months. The fish growth and optimum feeding rate is given in the Table.1.

Cage structure management

A minimum of one year to obtain marketable size fish is involved in the cage culture of orange spotted grouper. Hence, the cage structure should be sturdy and well designed. Some of the activities involved in cage management are net exchange, cage frame cleaning and checking of mooring. The cage net is prone to infestation with barnacles, algae and silt accumulation and adds extra weight to the nets. Hence, the net has to be changed periodically depending on their accumulation, varying with the seasons and locations. Based on the experiences from north Andhra coast, net exchange is required at least once in two months. If this is not done promptly, the net may tear off due to the load and it also impacts the buoyance of the cage frame. Cage frames which also act as a walkway are prone to the accumulation of barnacles that leads to tearing of the net ropes through rubbing and also reduces the durability of the frame. Hence it requires monthly cleaning. Cage mooring which keeps the entire cage structure in position, requires monitoring of the mooring chain, at least once in a month. The mooring system specified for the cages last for a minimum period of two years, and then needs to be changed based on the conditions of the chain.

Table 1. Feeding Schedule in grow-out system.

Days of Culture (DOC)	Size (g)	Feed Size (mm)	Feeding Rate (@ %body weight)		Feeding Frequency (per day)
			Artificial feed (A.F)	A.F + Low value fish	
0-60	20-75	1.8 to 3.0	8%	4% + 5%	4
60-120	75-150	3.0 to 5.0	6-5%	3% + 5%	4
120-180	150-275	5.0 to 6.0	5-4%	2% + 5%	2
180-240	275-450	6.0 to 1.0	4%	2% + 5%	2
240-300	450-650	1.0 to 1.5	3-2.5%	1% + 5%	2
300-360	650-800	1.5 to 1.8	2%	1% + 5%	2
360-450	800-1500	1.5 to 1.8	1.5%	0.5%+5%	2

Cage maintenance – Process of net exchange

Cage maintenance – Cleaning of cage frame

Fish health management

The cage cultured fish should be periodically checked for its feeding and health status, and should be sampled fortnightly. Apart from critical monitoring while sampling, daily observation of their feeding behaviour, which is a good indicator for the health status of the fish, is required. Three major disease-causing agents (parasites, bacteria and virus) that are mostly responsible for the diseases in orange spotted grouper culture systems and their controlling measures are given in Table 2. All diseases are associated with stress and the stressed fish are easily affected by the pathogens. Therefore, stress during culture should be minimised by maintaining good water quality, optimum feeding and stocking density. Among all, the virus infection can occur from hatchery produced larvae itself, so selecting an active and disease free larvae is an important measure to control the infection.

Fish harvest

Cage cultured fish being in a small confined environment, harvesting is easier than any other culture methods. Mostly the fish remains at the bottom and hence, during the harvest, the inner cage net should be lifted from all four sides and hung from hand rails of the cage frame. The fishes in the inner net are the harvested with the help of

Table 2. Important disease and their control in cage culture

Disease causing agents	Major groups	Symptoms	Control measures
Parasites	Protozoa, Monogena and Digenea	Skin irritation and rubbing against the hard surface and skin ulceration	Freshwater dip treatment
Bacteria	*Vibrio parahaemolyticus*, *V..alginolyticus*, *Streptococcus* and *Flexibacteria*	Haemorrhage, weakness, surface swimming and fin rot	Use of probiotics
Virus	Nidovirus and Iridovirus	Dark colouration, loss of equilibrium and mass mortality	Selection of disease-free juvenile fish

Harvest of orange spotted grouper from sea cages and transportation to shore in boats

Harvested orange spotted grouper with ice packing

a hand scoop net. Immediately after harvest, washing in clean water and chill killing is suggested to maintain the freshness, colour and quality of the harvested fish. These are packed in plastic trays or thermocol boxes by adding layers of ice in equal quantities at the bottom and top of the fish. Harvest of the fish in the early morning hours helps to maintain their freshness.

Fish marketing

The fish is highly popular in international trade in live and chilled conditions with Southeast Asian countries and United Arab Emirates (UAE) being the major markets. For live fish trade in Southeast Asian countries, premium price that is 3-4 time higher than the price of dead fish can be got. Chilled fish is another major mode of export of bigger sized fish each 1.5 – 2.0 kg, especially UAE. Colour of the fish plays an important role in marketing with pale white with clear orange spot fetching higher price than the black coloured fish with orange spots. Therefore, during harvest, minimum stress and services of a fish cold chain should be ensured.

Table 3. Economics of farming orange spotted grouper in marine cages

Expenditure item	Cost in ₹ (in lakhs)
Depreciation value on cage and accessories with an average life of 10 years for cage frame and five years for cage mooring and nets	4.3 (Cost of cage and accessories including installation: ₹300,000 per unit and depreciation is ₹43,000 per unit per year)
Operational expenditure	
Cost of 25000 numbers of grouper seeds of 20 g in size	5.0 @ ₹20 per seed (including nursery rearing expenses)
Artificial feed: Cost for 35.0 tonnes of extruded pelleted feed (Survival 80%; Average Body Weight 1 kg at harvest) at FCR 1:1.75 at ₹100 per kg of feed	35.00
Low value fish feed: Cost for 100 t of low cost fish feeds (Tilapia/trash fish) (Survival 80%; Average Body Weight 1000 g at harvest) at FCR 1:5.0 at ₹30.0 per kg of fish feed : total cost ₹30.0 lakhs per annum Additional expenditure towards preserving and processing the low value fish feed (Freezer, electricity and cutting machine) at ₹2.0 lakhs per annum (Cost for feed and feed maintenance)	32.0
Labour Charges at ₹36000 per month for 12 months (@ ₹10,000 per person for three person)	3.60
Boat hiring and Fuel charges at ₹6000 per month for 12 months	0.72
Charges for net exchange at ₹500 per person for 3 persons, five times in the production cycle for each cage	0.75
Miscellaneous expenditure feed medicines and probiotics	0.5
Expenditure (Sl No: 1-7)	49.87 (with Artificial feed) & 46.87 (with low cost fish feed)
Total income: Production: 20 tonnes at 80% survival with harvest size of 1.0 kg @ average selling price at ₹300 per kg	60.00
Net profit : (8-7) (lakh₹)	10.13-13.13

Economics

The total operational expenditure and profit for culture of the fish in a battery of 10 cages is given in the Table 3. Culturing the fish for 12 months at the stocking of 20 per m^3 will support the farmer with minimum net profit of approximately ₹10 lakhs with price realization of ₹300 per kg of fish. An additional ₹3 lakh could be earned by using low cost fishes as feed instead of artificial fish feed. However, the profit may vary depending on the market price of the low value fishes. Also, use of the low value fish feed is laborious as it requires fish storage and fish cutting before feeding. Continuous availability of the low value fish feed is uncertain due to increasing demand and frequent changes in availability.

The Best Management Practices (BMP) for grow out culture of orange spotted grouper can be summarised as below:

1. Periodical fortnight grading during nursery rearing is essential to reduce cannibalism.
2. Cages should be installed where water movement ensures optimum concentration of dissolved oxygen
3. Fish fingerlings of > 20g should be stocked for maximum survival
4. Cage net depth of 2.0 m should be maintained till the fish reach 250g
5. Inner cage net should be additionally supported with a middle ballast pipe for maintaining the round shape and avoiding folding of the net.
6. Low value fish feed should be given along with artificial pelleted feed for better growth and to avoid size variations
7. Periodical monitoring of fish, cage net and other cage system
8. Stress should be avoided while harvesting and the harvested fish should be maintained in ice till packing.

Ethics and humane practices of bleeding and euthanasia for experimental marine fishes in fish nutrition research

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Scientific research in fish consisting haematological, biochemical, bacteriological, parasitological, toxicological, immunological, somatic measurements, tagging, digital photography for morphometrics and reproductive investigations requires anaesthetization. An overdose of anaesthetic is also an effective method to euthanize fish humanely whenever necessary. The recommendations of universal ethical committee mentions that the fish should be relieved of pain (analgesia) through block of pain perception with or without the retention of other sensory abilities while collecting the blood or euthanasia.

Anaesthetization

Sedation is a preliminary state of anaesthetization and on continuation drowsiness is induced, with dulled sensory perception and with some analgesic effect (insensitivity to pain), but no gross loss of sensory perception or equilibrium. In fish, the light and deeper sedation has been identified by responsive to stimuli through reduced motion and ventilation. The progressive stages of sedation is called anaesthesia with three obvious phases such as induction, maintenance and recovery. The light anaesthetic response can be defined as partial loss of equilibrium and good analgesic effect and the deeper anaesthesia is indicated through total loss of muscle tone, total loss of equilibrium and almost nil ventilation. The last stage of anaesthesia is called as medullary collapse in which ventilation ceases, cardiac arrest and eventual death that occurs through overdose either through prolonged exposure of higher anaesthesia concentration (McFarland, 1959).

For euthanasia, fish may be given appropriate dose and duration of deeper anaesthesia to give complete pain relief. Therefore, for each fish the anaesthesia should be selected appropriately and dose should be optimized to get better effect on induction, maintenance and recovery of anaesthesia. The induction phase should provide quick ataxia (loss of equilibrium) and loss of the righting reflex and the maintenance phase involves extending the achieved state of anaesthetic effect in a stable manner without detriment to the health of the fish. The recovery phase involves withdrawal of the anaesthetic agent and return to a normal state in time duration of few seconds to a few minutes, without altered behaviour or side-effects. Among the several anaesthetic agents, tricaine methanesulfonate (MS222) and clove oil are very safe and does not have many side effects in marine fishes.

Blood collection in marine fishes

Several methods such as caudal venous puncture, dorsal aorta puncture, tail ablation, decapitation, severance of caudal vein and heart puncture (MUAWC, 2008) are available. The blood collection technique is chosen based on several factors such as the size, health status, the quantity of blood required and the fate of the fish (sacrificed or rescued) in a study. Most of these methods are destructive as fish may be killed during blood collection. While collecting blood, if the fish becomes too much stressed it can compromise the parameters studied. In caudal venous puncture method, the fish can recover to its original health within a few days post blood collection. Therefore, marine fishes such as snubnose pompano, Asian sea bass, orange spotted grouper, cobia and mangrove red snapper are subjected to standardized caudal venous puncture method for fish nutritional research. In general, the volume of blood in most of the fish is about 6% of its body weight and hence, for any serial blood collection study, the blood should not be withdrawn beyond 1/6th of the total volume available in the fish.

In ICAR-CMFRI the anaesthetization protocol was standardized using clove oil and MS222 for snubnose pompano and orange spotted grouper. For blood collection, the is transferred carefully and with least stress into the vessel with anaesthetic agent. The fish is allowed to swim until with ataxia, it loses its equilibrium and gets fully anaesthetized. When it completely loses its equilibrium, it should be taken out and kept in soft wet cotton cloth to prevent mucous sloughing during sampling. It should be ensured that the fish does not show any movement (opercular movement and muscle tone) and therefore does not feel pain during the blood collection process.

The needle and syringe should be selected according to the size of the fish (Table 1). The needle should be sterile and changed for every fish to avert blood clotting while collecting blood. For small size fish, 26 G needle fixed in 1 mL tuberculin syringe is ideal and as the size of the fish increases, the needle and syringe size should be selected. The needle should be rinsed with anticoagulant (2.75% EDTA solution or heparin) to avert blood coagulation during the course of blood collection. The needle is inserted beneath the scales in the caudal peduncle area, just below the lateral line at about 45° angle to the lateral surface in a cranial or ventral direction, until it touches the vertebral column. This can be felt by hard impenetrable surface and thereafter, the syringe is withdrawn slightly, approximately 1-2 mm, so that the blood vessel beneath the vertebral column can be pierced and sampled. After blood collection, the fish is released into a recovery tank with freshly aerated aesthesia-free water and after the anaesthetic effects are withdrawn the fish starts to swim freely.

Blood collection from snubnose pompano by caudal venous puncture

Plasma and serum collection and storage

For the preparation of plasma, the collected blood is transferred carefully into anticoagulant coated vacutainer collection vials [EDTA-treated (lavender tops); citrate-treated (light blue tops); heparinized tubes (green tops) or sodium fluoride tainted (grey top)] rapidly by removing the needle to prevent haemolysis. The blood is mixed gently with anticoagulant in the container and should be used for further analysis within 3 hours at room temperature. It can be stored for 24 hours at refrigerated temperature (4 °C). The plasma is collected by removing blood corpuscles through centrifugation at 5000 rpm for 10 minutes in a refrigerated centrifuge. The supernatant plasma can be preserved at 2–8°C for few days and at –20°C for prolonged period, without much freeze-thaw cycles. For serum collection, the blood should be released into the anticoagulant free container (red top) and kept in slanting position. The blood is allowed to clot by leaving it undisturbed at room temperature for 2 hours for better separation of serum. The clot portion is removed by centrifugation at 5000 rpm for 10 minutes in a refrigerated centrifuge. The resulting supernatant serum is transferred into a clean polypropylene tube. The

Table 1. Dimensions of needle and syringe in relation to fish size

Fish size	Needle Gauge	Outer diameter (mm)	Inner diameter (mm)	Syringe
<25 g	26 G	0.46	0.26	1 mL
25-100 g	24 G	0.57	0.31	1 mL / 2 mL
100-500 g	22 G	0.72	0.41	2 mL
0.5-2 kg	20 G	0.91	0.60	5 mL

serum samples can be stored for 7-10 days at 2–8°C. If the serum is not analyzed immediately, it should be apportioned into 0.5 mL aliquots and stored at –20 °C until analysis not exceeding 3 months. It is advisable to avoid frequent freeze-thaw cycles because this is detrimental to many serum components.

Euthanasia is generally practiced to collect the tissue samples from the experimental fishes by sacrificing them after completion of experiments to assess the enzymological, biochemical, immunological assays and reproductive performances. In such situation the fish should be killed humanely, without giving any pain, that can be achieved through overdosed anaesthetization (Table 2).

Table 2. Dosage of anaesthetics commonly used for anaesthesia and euthanasia in fish

Anaesthetic	Dosage*	
	For anaesthesia	For euthanasia
MS-222	75-150 mg L^{-1}	1 g L
Clove oil	0.2-0.5 mL L^{-1}	> 1.5 mL L^{-1}
Benzocaine	100 mg L^{-1}	-
Etomidate	1-4 mg L^{-1}	-

*Dosage may vary with species, age and size of fish

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Brief Communications

Length-weight relationships of *Meretrix casta* in estuaries of north Kerala

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The yellow clam *Meretrix casta* (Chemnitz, 1782) forms significant sustenance level fisheries as it is used for local consumption as well as for the lime industry. Length–weight relationships of *M. casta* from Chaliyar, Moorad estuaries (Kozhikode district), Kavvai estuary (Kasaragod district) and Mahe estuary, Mahe are reported as they are important inputs in fisheries management decisions.

The total length of the clams were measured using digital vernier calipers to the nearest 0.1 mm along the antero-posterior axis and width along the dorso-ventral axis. The maximum distance between the valves when they are closed was considered as height. The total weight, wet meat weight and shell weight were recorded to the nearest 0.1 g.The length-weight relationship (LWR) was determined by the equation $W = aL^b$. The parameters, *a* and *b* were estimated by linear regression analysis (least squares method) and 95% confidence limits of b and the significance level of R^2 were also estimated. Length-weight relationships for *M. casta* from four different estuaries indicated there is no significant difference between males and females therefore a combined equation was derived (Table 1).

M. casta from all the four estuaries exhibit isometric growth. *M. casta* from Moorad estuary is heavily exploited, with mean length at 22.7 mm, clams less than 10 mm are being fished. Clams from Chaliyar estuary were also heavily exploited, with mean length at 21.5 mm with clams of 15 -33 mm being fished. *M. casta* from Kavvai estuary is moderately exploited, with mean length at 21 mm with baby clams also exploited and sizes of 11 -38 mm being fished. *M. casta* from Mahe estuary is not exploited, with mean length at 27 mm. *M. casta* is fished

Table 1. Length-weight relationships for *M.casta* in various estuaries

Estuary/ Period of Study	Minimum Size mm	Maximum Size mm	Length –Weight Relationship	N	r^2
Moorad, Kozhikode / 2008	9.2	39.1	Log Y = -0.981 + 2.919Log X	623	0.8973
Chaliyar, Kozhikode / 2008	15.8	33.4	Log Y = -1.259+ 3.09Log X	1560	0.9030
Kavvai, Kasargod / 2008	10.8	37.8	Log Y = -0.987 + 3.054Log X	1559	0.6137
Mahe, Pondicherry / 2006-2007	20	38	Log Y = -3.419 + 3.069Log X	550	0.5743

regularly in most estuaries mostly for local consumption. However, in Moorad estuary the exploitation is intense and clams less than 10 mm are also exploited. It is necessary to implement regulatory measures to regulate the exploitation of baby clams by implementing the Minimum Legal Size (MLS) of exploitation. Re-laying of baby clams in suitable areas can ensure the utilization of the juvenile clams exploited during peak seasons, so as to harvest later at bigger sizes to enhance production and sustain the stock. This method has been very successful in the case of the black clam in the Vembanad Lake recently. The possibility of exporting the meat and production of value added products also needs to be explored.

Brief Communications

Economic empowerment of SHGs through Pearlspot Seed Production Technology

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Pearlspot being recognized as the state fish of Kerala. The seed production technology of Pearl spot ensures an avenue for the livelihood enhancement of fisherfolk through farming. The ICAR-Central Marine Fisheries Research Institute (CMFRI), plays a vital role in disseminating the seed as a part of the project entitled "Empowerment of Scheduled Caste Fisherfolk through Entrepreneurial Capacity Building of Self-Help Groups in Marine Sector" funded by the Department of Science & Technology (DST), New Delhi under the Scheduled Caste Sub-Plan (SCSP) programme setup a Pearl spot seed production unit in Vallarpadom, Ernakulam district, Kerala. Self Help Groups (SHGs) were mobilized with the assistance of the Krishi Vigyan Kendra (KVK) of ICAR-CMFRI and women participation as the essence of the gender mainstreaming was ensured.

A pragmatic and utilitarian combination of extension research and practical extension was adopted for conducting this study. The project team of ICAR-CMFRI visited the Vallarpadom location twice a month for two years and conducted communication conclaves and interaction programmes for the fisherfolk on Pearlspot seed production units. A series of farmer collaboration conclaves were organized for these SHGs. The technical assistance was provided by the professionals from KVK of ICAR-CMFRI, who played a fundamental role in the practical implementation of the project. Interaction meetings for imparting awareness among fisherfolk beneficiaries were organized on the site, and training programmes, including broodstock release and feeding, were successfully carried out. A training pamphlet in vernacular was distributed to the SHG members as a ready reckoner for convincing the seed production

Pearlspot seed production unit operating in Vallarpadam

technology of pearl spot.

A pond of 50 cent area was prepared primarily, and 200 pearl spot brooders were stocked in the pond and looked after till the fish attained appropriate marketable size within a couple of months. The linkage established gave the benefit of the provision of a pump set from KVK of ICAR-CMFRI. Stage by stage video documentation in the various segments of activities of SHG in seed production was done. The practical extension part for the present study consisted of Awareness & Entrepreneurial Capacity Building (ECB) Training programmes systematically executed, and then the extension research part focused on socio-economic surveys with a pre-tested and structured data gathering protocol with standardized scales and indices. The participation profile, decision making, gender need analysis, economic feasibility analysis, assessment of performance level of SHG, empowerment index calculation etc., were undertaken.

The male and female counterparts of the families were separately interviewed to assess the gender mainstreaming aspects in terms of equity and equality for access to resources, participation profile, decision making aspects, gender need analysis etc. The extent of involvement in various phases of the entrepreneurial activity was

Training of SHG for Pearlspot seed production

Pearlspot seed collection for marketing

quantified and expressed. Maximum participation of the members and families was observed during pond preparation, fertilization of pond, feeding, maintenance of juveniles, oxygen filling and packing of fish seed, marketing, account and record-keeping, arrangement of other inputs, etc. Though the majority of activities such as pond preparation, fertilization of the pond, broodstock collection/purchase, transportation of broodstock, the introduction of broodstock, fixing of egg-laying surfaces and water management are male-dominated, the female counterparts of the households also played a definite role in the activities like feeding, maintenance of juveniles, oxygen filling and packing of fish seed, marketing, account and record-keeping and arrangement of other inputs. The opinions of men and women in the above aspects were found to be similar without any significant difference. However, differential gender response was observed among SHGs.

The Economic Feasibility Analysis of the Pearlspot seed production units of SHGs was undertaken using the data collected for the last four years on cost and earnings of the farming activities and by using indicative economics. The economic feasibility indicators such as average operating cost, average net returns, break-even point (BEP) and payback period (PBP) of these enterprises were worked out. For a production unit of 50 cent, in 12 months, the Break-Even Point was estimated to be 8,008 seeds at the rate of ₹10 per seed. The payback period, the amount of time taken to recover the cost of investment of the venture, was computed as within one year. The enthusiasm exhibited by the Scheduled Caste fisherfolk boosted their confidence to such an extent that they enhanced the seed production unit further to 2 acres. The harvest results brought bumper output, and the juveniles were sold at ₹11.50 per piece. The first sale was accomplished amid the acute COVID-19 pandemic and associated lockdown period. The social and economic empowerment dimensions and capacity building aspects achieved the highest score in the Empowerment Index analysis. The Benefit-Cost Ratio for the pearl spot seed production technology was 2.5. Two more SHGs have established similar seed production units as subsequent ventures under the DST project in Narakkal and Karumaloor locations. The success case study elucidated can be used as case model and practical manual for promoting group action for mobilizing SHGs on a sustainable basis.

The commercial viability of the seed production unit of Pearlspot, the State fish of Kerala is a major aspect determining its adoption in the selected location. The seed production technology of pearl spot is found to be economically viable, technically feasible, ecologically sound, environmentally friendly, socially acceptable and hence a sustainable one. The technology can play a significant role in enhancing fish production stock replenishment, providing an alternative livelihood option, creating employment opportunities for fisher youth, improving the fishers' income and effective utilization of hitherto underutilized water resources. The documentary can be used as manual for mobilizing similar SHG ventures on a sustainable basis.

Brief Communications

A report on egg mass of Hound needle fish *Tylosurus crocodilus*

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During an oceanographic cruise of ICAR-CMFRI on board FV Silver Pompano on November 2021, a bunch of pale-yellow spherical egg consisting of about thousand numbers connected by strong filaments on the chorion

Fig. 1. Bunch of Hound needle fish eggs.

Fig. 2. Eggs chorion with tendrils

were obtained while undertaking experimental trawling in off Kochi region at 20-meter depth (Fig 1). After taking photographs and enumeration of the egg bunch, a small sub sample was taken for further analysis and the remaining eggs were released back into the sea immediately. The collected egg samples were preserved in 95% ethanol and brought to lab for further analysis. Preserved eggs were observed and photographed in the lab by Nikon SMZ 25 stereo zoom microscope. The eggs were small, round (2.5- 2.9 mm diameter) and tanned with eyed embryo having pigment spots on head, yolk sac and tail. Pigmented eyes and nares observed, oil globule was absent and chorion was armed with numerous long filaments to keep them attached. These strong filaments (tendrils) were interwoven with other egg filaments and braided in to a bunch. Detachment of eggs from the bunch was difficult because they were strongly interwoven with filaments. For confirming the identity, isolation of genomic DNA from the egg was done following phenol chloroform method (Sambrook, J., 2006). The COI gene was amplified using primers Fish F1 5'-TCAACCAACCACAAAGACATTGGCAC-3' and Fish R1 5'-TAGACTTCTGGGTGGCCAAAGAATCA-3' (Ward *et al*. 2005) using standard protocols. After sequencing and alignment, we got 683bp sequence. Similarity search (BLAST) in NCBI database showed 100% identity to a needle fish species, *Tylosurus crocodilus* with 100% query coverage. The sequence was submitted to NCBI Genbank with accession no: SUB 11374388 HFE ON331773.

Tylosurus crocodilus (Peron and Lesuer, 1821) commonly known as Hound needle fish (family Belonidae) is a commercially important needle fish (pelagic) distributed in tropical and subtropical oceanic waters, they normally prefer warmer temperatures of about 20-30°C. The most common depth of occurrence reported for the species is between 0-13m (Breder and Rosen, 1966). Six species of needle fishes namely: *Tylosurus crocodilus*, *Tylosurus acus melanotus*, *Strongylura strongylura*, *Strongylura leiura, Ablennes hians* and *Xenentodon cancila* have been reported from south west coast of India (Rema Devi *et al*., 2013; Barman *et al*., 2013; Roul *et al*., 2018). It is reported that epipelagic eggs of *Tylosurus crocodilus* is found attached to objects in the water by tendrils on the egg's surface (Breder and Rosen, 1996). Physico-chemical parameters of the water from the site was analysed as per the standard methods (APHA, 1998). The mean water temperature, pH, salinity and dissolved oxygen at the station was 28° C, 8.03, 31 PSU and 5.4 mg L^{-1} respectively. Transparency of water was 3.4 m, Chlorophyll a content was 0.2 mg/m^3 and turbidity was 2.2 NTU. Zooplankton analysis shows the abundance of copepods and hydrozoa, with 1425.5 and 61.5 numbers per cubic metre respectively.

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Harvesting of *Nannochloropsis oculata* by chemical flocculation

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Microalgae culture forms an inevitable component in aquaculture and mass culture on a commercial scale is essential to satisfy requirement in the hatchery for use as functional foods and nutraceuticals. However, the current microalgal production technologies are not cost-effective and face several bottlenecks, among which is the harvesting of microalgal biomass. Typical strategies currently applied for harvesting microalgae include centrifugation, filtration, various forms of flocculation (e.g., chemical inorganic and organic agents, alkaline flocculation, bio-flocculation using microorganisms, and electro-coagulation), sedimentation, and flotation. Among these harvesting methods, flocculation combined with sedimentation of microalgal flocs is considered best with reported cell recovery of > 90% and with low cost. However, the biomass thus recovered with chemical flocculant may cause harm to the final product but it is still regarded as a promising technique. In the present study, the flocculation efficiency of $ZnCl_2$ and $ZnSO_4$ were tested as chemical flocculants for the harvest of *Nannochloropsis oculata*.

Marine microalgae, *Nannochloropsis oculata* culture was used as the culture inoculum. The algae was cultured in photobioreactor (Celeritus Engineering, Ahmedabad) with Conway medium at temperature of 18-21^{0}C, pH of 7.8-8.4, salinity of 23-25 ppt and light intensity of 2000 lux. Flocculation experiments were carried out in stationary phase cultures (28 million / ml). 900 ml of the culture was taken in 1000 ml glass beaker and different concentrations of flocculants were added. Based on our previous experiment on electro flocculation study, chemical compound with Zinc was considered. Two different flocculants were used; $ZnSO_4$, and $ZnCl_2$ at varying concentration ranging from 0.2 g/L to 1 g/L. The flocculation efficiency was measured after 4 hours from all the experiments which were done in triplicates. The initial microalgal biomass concentration in the beaker was estimated from the optical density of 750 nm. After 240 minutes, the optical density of the supernatant was measured at half the height of the clarified culture. Culture broth containing no flocculant was used as control. Flocculation efficiency was calculated using the formula:

Flocculation efficiency (%) = (1–A/B) x 100; Where, A= OD value of sample at 750 nm and B = OD value of control at 750 nm

$ZnSO_4$ with 0.8g/L performed maximum flocculation efficiency of 92.54 % followed by 0.2g/L (71.79 %). Least efficiency was recorded in 1.0 g/L which was similar ($p>0.05$) to 0.4g/L. Whereas, with various concentration of $ZnCl_2$, the harvesting efficiency was varied from 70.55 %–77.54 %. The highest efficiency was registered with 0.4 g/L of $ZnCl_2$ followed by 0.2 and 0.6 g/L. Not much difference ($p>0.05$) was observed between 0.2 and 0.6 g/L. Lowest harvesting efficiency (70.55%) was recorded in $ZnCl_2$ with concentration of 1.0g/L.

Evan's Blue stain was used for testing the cell viability of the flocculated *Nannochloropis* cells. For staining, a 20 mL sample of flocculated *Nannochloropis* was treated with 1 ml of 1% (w/v) stock solution of Evan's Blue. The samples were allowed to stand at room temperature for a minimum of thirty minutes before microscopic examination. A subsample of each stained suspension was then inspected at 40 X magnification using an

Improved Neubauer Haemocytometer (Superior Co., Berlin, Germany). ZnSO4 (0.8%) performed better with superior viability compared to other concentrations (0.2, 0.4, 0.6 &1.0g/L). During the Evan's blue staining, most of the *Nannochloropsis* cells were greenish in colour and hence, were not stained (80% viability). *Nannochloropsis* cells flocculated with various $ZnCl_2$ concentrations had white precipitations. However, after staining with Evan's blue, the cells were greenish and individually dispersed (>40% viable cells).

Pre-treated sea water passing through slow sand filter, UV filter and further treated with ozone was used for the inoculation of flocculated microalgae. The flocculated microalgal cells to be used as inoculum were diluted and mixed properly with the help of magnetic stirrer to ensure the uniform distribution of individual cells. The initial cell count was maintained at $5x10^5$ cells/ml. These were cultured at temperature of 18-21^{0}C, pH of 7.8-8.4, salinity of 23-25 ppt and light intensity of 2000 lux with Conway medium. Cell count was estimated after 7 days of inoculation. *Nannochloropsis* flocculated with 0.8g/L $ZnSO_4$ could produce maximum cell count of $9x10^6$ cells/ml. The rest of the concentrates, with other concentrations of $ZnSO_4$, also could perform, albeit with less number of cell counts. Whereas, *Nannochloropsis* cells flocculated with $ZnCl_2$ took more time to increase the cell count, and among various concentrations of $ZnCl_2$, least concentration performed better (0.2g/L). It is concluded that, chemical flocculation of *Nannochloropsis oculata* with 0.8 g/L $ZnSO_4$ may be an effective method for harvesting of large volume of microalgae culture with good harvesting efficiency. Further studies are required for its commercial applications in various industries and fish hatcheries.

Brief Communications

Report of two surgeonfishes, *Naso reticulatus* and *Naso lopezi* from coastal waters of Maharashtra, Arabian Sea

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Fishes of the family Acanthuridae (Perciformes), called Surgeonfish, tang, and unicorn fish are common marine fishes observed in tropical and subtropical waters, with 85 valid species reported in 6 genera (Fricke *et al.*, 2022). In Indian waters, Mohapatra *et al.*, (2020) listed, 33 species of surgeonfishes under 5 genera, however, missed 2 species listed by Nair *et al*. (2014), ie *Naso lopezi* Herre 1927 and *Naso thynnoides* (Cuvier 1829), and another 2 species listed by Rajan *et al.* (2013) from Andaman waters ie, *Acanthurus japonicus* (Schmidt 1931) and *Zebrasoma velifer* (Bloch 1795), making the total surgeon fishes known from India to 37. Acanthurid fishes are known for long larval durations and widespread population connectivity. However, interestingly new geographical records are continuously reported for several species in the Acanthuridae from the commercial fishery along Indian coasts (Ray *et al*., 2013; Roul *et al.,* 2019) suggesting dedicated diversity studies. During regular fishery monitoring surveys in Maharashtra, two previously non-reported surgeonfishes were observed in the fishery landings. The fishes are identified as *Naso reticulatus* Randall, 2001 and *N. lopezi* following Randall (2001) which are not previously known from Maharashtra.

Naso reticulatus Randall, 2001

Reticulate Unicornfish

Naso reticulatus (53 cm TL) collected at Sasson dock Mumbai, Maharashtra

Naso reticulatus, is currently known from Pakistan to Indonesia. In India, Mohapatra *et al.* (2013) reported *N. reticulatus* from West Bengal and Roul *et al.* (2019) reported the species from Gujarat, Kerala, Tamil Nadu, Andhra Pradesh and suggesting the species is widely distributed rather than previously known. Since October 2021 it is landed in stray numbers in gillnet and trawl fisheries. *Naso reticulatus* is assessed as Data Deficient (DD) in the IUCN Red List of Threatened species.

Naso lopezi Herre, 1927

Elongate Unicornfish

*Naso lopezi (*55 cm TL) collected at Sasson dock Mumbai, Maharashtra

Naso lopezi was previously known from southern India, Maldives, Sri Lanka, to New Caledonia. In India, it is reported from Andaman waters (Rajan *et al.,* 2013) and Southern India. It is also figured in Nair *et al*. (2014). On 4th February, 2022 two specimens were landed at Sassoon dock, Mumbai by gillnetters operated off Dhabol, Ratnagiri in the Arabian Sea. This report extends the known distribution range of species to the northern Arabian Sea. *Naso lopezi* has been assessed as Least Concern (LC) on the IUCN Red List of Threatened Species.

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Stranding of dolphins along north Andhra Pradesh coast

Grampus griseus at Rushikonda beach, Visakhapatnam

Sousa chinensis at Sagarnagar area, Visakhapatnam

On 6th November 2021, a Risso's Dolphin, *Grampus griseus* (G. Cuvier, 1812) which measured as 230 cm in total length was stranded on the Rushikonda beach, Visakhapatnam. Reported stranding of this species from coast of Andhra Pradesh is rare. On 22nd September 2021, a Long-beaked common dolphin, *Delphinus capensis* Gray, 1828 was washed ashore at Kambalarayudupeta, Srikakulum. It measured 138 cm in total length and weighed approximately 38 kg and exact cause of the death was unclear. Similarly, Humpback dolphin, *Sousa chinensis* was recorded at Sagarnagar and Rushikonda beaches, Visakhapatnam on 8th April 2021 and 11th September 2021 respectively

Delphinus capensis at Kambalarayudupeta, Srikakulum

Sousa chinensis at Rushikonda beach, Visakhapatnam

(Reported by Pralaya Ranjan Behera*, Loveson L. Edward, H.M. Manas, Subhadeep Ghosh, M. Satish Kumar, and Ashok Maharshi | Visakhapatnam Regional Centre of ICAR-Central Marine Fisheries Research Institute)

Report on crested hair tail *Tentoriceps cristatus* (Trichiuridae) from Goa

A single specimen of ribbon fish *Tentoriceps cristatus* (Klunzinger, 1884), commonly called the crested hair tail, was landed in Cotbona jetty, South Goa on 26th November 2021. The specimen was collected from a mechanized purse seiner which operated at a depth of 60-80 meters off west of Cotbona. The mature female specimen weighed 119 g and was 71 cm in length. The species was found along with the commonly landed *Trichiurus lepturus* (large head hairtail). This is the first record of *T. cristatus* from Goa coast.

Morphological identification was based on the silvery elongate, strongly compressed and ribbon like bodywith dorsal side of head convex shaped. Eye, large and situated laterally. Large mouth with dermal flaps at tip of both jaws, front teeth without barbs. Convex hind gill margin. Single long dorsal fin, pectoral fins short and not reaching the lateral line, caudal fin absent.

Molecular identification was conducted with the tissue samples collected and preserved in 95% ethanol followed by isolation of DNA with the Phenol Chloroform method. The COI gene was amplified using primers Fish F1 and Fish R1 using standard protocols. After sequencing and alignment, 683bp sequence was obtained. Similarity search (BLAST) in NCBI database showed 99.85% identity to *T. cristatus* with 99% query coverage. The sequence was submitted to NCBI Genbank with accession number: ON183167.

Reported by A. Anuraj*, A.H.Kamath, N. S. Jeena, K. A. Sajeela, Mahendra Pal, P. P. Suresh Babu and Boby Ignatius | Karwar Regional Station of ICAR-Central Marine Fisheries Research Institute

The megamouth shark reported from Indian waters

The first confirmed report of *Megachasma pelagios* (Lamniformes: Megachasmidae) from Indian waters based on a juvenile male shark that landed at Thengaipattinam landing centre, Tamil Nadu, India on 25th April 2022 by a multiday gillnetter cum longliner operated off south of Thengapattinam is given. The shark measured 280 cm in total length and weighed 48 kg. The Megamouthshark, *M. pelagios* (Lamniformes: Megachasmidae) is reported to have maximum size of 820 cm TL. It was described as a new species in 1983 and around 261 specimens have been documented till 2021 with most of the reports from Northwest and Western Central Pacific. The Indian Ocean records are widely scattered and mostly reported from South Africa, Sri Lanka, Thailand, Indonesia and Australia. Mr. Jikson Jeromeraj of ICAR-CMFRI stakeholder network shared the photographs of the megamouth shark.

Megachasma pelagios landed at Thengaipattinam Fisheries Harbour

Reported by S. Surya*, K.V. Akhilesh, J.H. Kingsly, G. Angel, D. Dispin, V.A. Leslie, I. Albert, K.K. Suresh, E.M. Abdussamad and M.K. Anil | Vizhinjam Regional Centre of ICAR-Central Marine Fisheries Research Institute

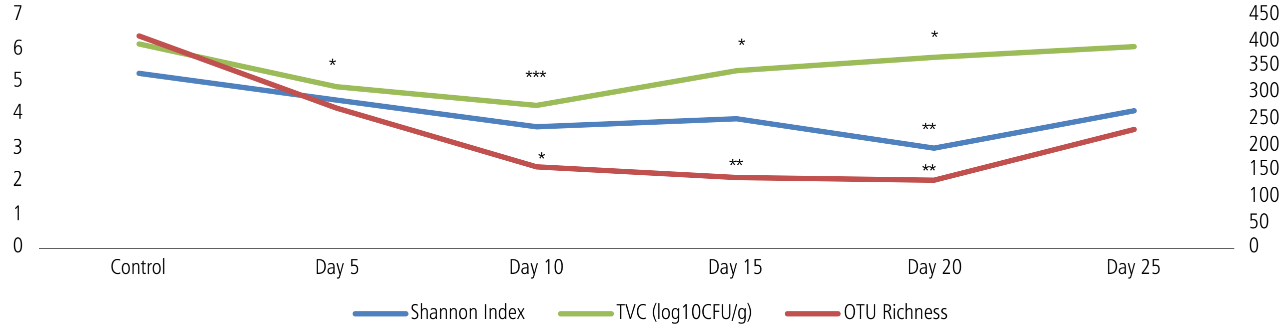
Safety guidelines for aquaculture in response to antibiotic treatment-induced changes in the fish gut microbes

Antibiotics have been used in aquaculture health management protocols, but whenever used without adequate precautions, it can lead to serious repercussions including drug accumulation, drug resistance and distortion of a beneficial, natural intestinal microbial fauna. Microbial flora associated with fish guts have a key role in digestive functions and development of the immune system. Hence good aquaculture practices and disease prevention protocols in the aquaculture industry should be based on scientific facts. Both environment and host-associated factors are reported to influence the fish gut microbiome and the challenge is to fully understand these complex relationships, with respect to specific species of aquaculture importance.

[1]**T. G. Sumithra, K. S. R. Sharma, G. Suja, S. Gayathri, P. Vishnu, P. V. Amala, P. Sayooj, P. Ambarish, M. K. Anil, P.K. Patil and A. Gopalakrishnan. 2022. Dysbiosis and restoration dynamics of the gut microbiome following therapeutic exposure to Florfenicol in Snubnose pompano (*Trachinotus blochii*) to aid in sustainable aquaculture technologies.** | *Frontiers in Microbiology*, 13: 1-17, DOI= https://doi.org/10.3389/fmicb.2022.881275

The discovery of antibiotics is one of the most significant medical advances of the 20th century. Increasing reliance on farmed fish for human nutrition has led adoption of intensive farming practices which has spurred disease outbreaks in aquaculture systems. The aquaculture farmers are hence compelled to use antibiotics under controlled conditions to control fish mortality events. Antibiotics have played a significant role in treating and controlling bacterial diseases, which are serious issues affecting aquaculture operation and economic returns to farmers. The antibiotic treatment is crucial in fish farming in India, where prophylactic strategies using vaccines are completely absent. There are only four recommended antimicrobial compounds for aquaculture purposes, *viz*. sulfamerazine, oxytetracycline, sulfadimethoxine-ormetoprim, and florfenicol. The research carried out under the funding of Indian Council of Agricultural Research (ICAR) All India Network Project on Fish Health, Grant No. CIBA/AINP-FH/2015–16) has highlighted the need for implementing certain safety guidelines in aquaculture practices to ensure animal, human and environmental health during antibiotic applications[1].

Gut microbes play a major role in the health and disease of humans and various animals. Studies in terrestrial animals, including humans, have demonstrated the wide-ranging health implications of antibiotic treatment due to the changes in the gut microbes, while similar studies in fishes are scarce. The effect of antibiotic treatment on the gut microbiome of marine fish has rarely been considered so far. In this context, a study on the changes in gut microbes induced by the recommended therapeutic dose of florfenicol (FFC) in a marine aquaculture species, silver pompano (*Trachinotus blochii)* was done by ICAR-CMFRI. The experimental fish with FFC medicated feed @ 10 mg/kg biomass for ten days followed by non-medicated feed and monitored for 30 days. The control group were fed with non-medicated feed throughout the experiments. Fish from both groups were randomly sampled at five-day intervals to study gut microbial changes with scientific



Dynamics of gut microbes following FFC treatment

OUT richness depicted on the right side Y-axis. TVC & Shannon index depicted on left side Y-axis.
TVC: Total Viable Count; OTU: Operational taxonomic units; CFU: Colony Forming Units

tools involving culture-dependent and culture-independent methodologies.

Results

FFC treatment significantly reduced the counts, diversity, and richness measures of the gut microbes.

There was a complete restoration of the gut microbes (in terms of cultivable bacterial load, diversity measures of metagenomics, functional metagenomic profiles, and taxonomic composition up to class level) within 10-15 days after the treatment.

FFC therapeutic exposure induced significant short-term changes to the gut microbes, which were restored to the control level at 15 days post-withdrawal. The changes in gut microbial composition which were studied in detail using metagenomic data analysis tools revealed significant changes in the relative abundance of several gut microbes.

Most importantly, there was a transient rise in the relative abundance of certain opportunistic fish pathogens belonging to *Vibrio* sp., *Enterovibrio* sp., *Photobacterium* sp., *Pseudomonas* sp., and *Shewanella* sp. during 10 to 15 days post-withdrawal of FFC treatment. This suggests the possible increase in the susceptibility of the treated fish to different opportunistic diseases.

The gut microbial changes in terms of antimicrobial resistance indicated the therapeutic dose of FFC did not promote the FFC-resistant microbes in the gut of the treated fish. However a transient increase in kanamycin-resistant microbes and an abundance of multidrug resistance encoding genes during the initial ten days of post-withdrawal was recorded.

Highlights

Homeostasis of the gut microbes is transiently perturbed by the antibiotic treatment with a transient increase in the relative abundance of opportunistic pathogens. This can make the fish more prone to secondary infections during the withdrawal period. To deal with this, stress management measures like probiotics in aquaculture during the initial days of the antibiotic-treatment withdrawal period, is required.

The therapeutic dose of the particular antibiotic did not increase the likelihood of the same antibiotic resistance acquisition by the gut microbes, and hence the need to stick to the therapeutic dose and duration during treatment is important. However, there is a probability of the emergence of certain other antibiotic-resistant microbes as a secondary effect of the treatment.

The transient increase in the abundance of multidrug resistance encoding genes within the gut of the treated fish was observed. Hence, to avoid the probable emergence and dispersal of antimicrobial resistant bacteria in the aquatic environments, adequate measures for processing aquaculture effluents during the post-withdrawal period is required.

Science Corner

Post-transportation changes in the microbiome of cobia linked to increased disease susceptibility

Cobia (*Rachycentron canadum*) is a favored species for mariculture in India. However, seed production centres are not always located near farming sites, necessitating transportation of fish seed and juveniles used for stocking the open sea cages. Teleost gut microbiome is considered as a stress biomarker, which in aquaculture systems can be altered by poor water quality, dietary imbalances, pathogens in the environment, handling and transportation. Knowledge on changes in gut microbiome during transportation, can enable better fish health management strategies which in turn will enhance the profitability of the aquaculture sector.

[1]**T. G. Sumithra, S. Gayathri, S. R. Krupesha Sharma, Sanal Ebeneezar, K. K. Anikuttan, K. A. Sajina, G. Iyyapparaja Narasimapallavan, K. J. Reshma, R. Vishnu, G. Tamilmani, M. Sakthivel, P. Rameshkumar, D. Linga Prabu, P. Vijayagopal and A. Gopalakrishnan. 2022. Metagenomic signatures of transportation stress in the early life stages of cobia (*Rachycentron canadum*) to aid in mitigation strategies.** |*Aquaculture*, 559, 738407, https://doi.org/10.1016/j.aquaculture.2022.738407

Research on the microbiome of farmed fishes to explore the ways to achieve sustainable aquaculture production is a new arena in marine science. Knowledge of the microbial changes in different aquaculture operations which presently remains largely unknown, can reveal different beneficial microbial manipulation strategies for better fish health management. Transportation of live fish larvae and juveniles for routine aquaculture operation by the farmers causes multiple kinds of stress to the animals, resulting in mortality. In this context, the microbiome research was conducted under the Department of Biotechnology, Govt. of India funded "E.G. Silas Centre of Excellence and Innovation in Marine Fish Microbiome and Nutrigenomics (EGS–CoEI) "(BT/AAQ/3/SP28267/2018) project operated by ICAR-CMFRI. The team explored the identification of microbial changes induced by the live transportation of the larval and early-juvenile stages of cobia (*Rachycentron canadum*), a high-value marine aquaculture species. Transport conditions (~10 hours) without the application of

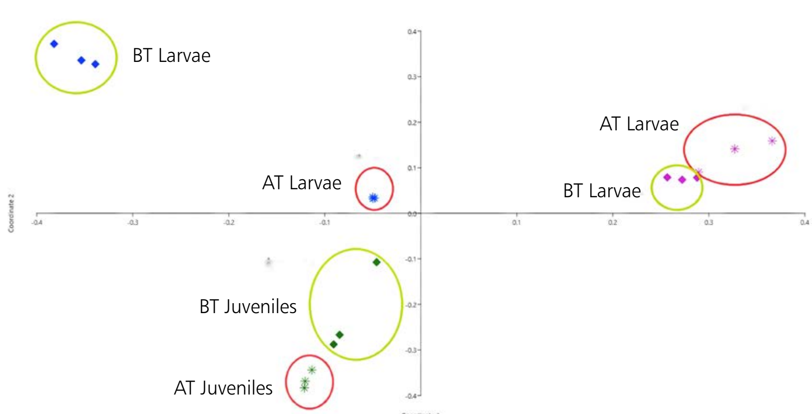


Fig. 1. Principal coordinate analysis on the microbiome profiles following transportation

any stress-relieving manipulations was taken as the standard. The juvenile gut samples and whole larval homogenates were randomly sampled before and after transportation, and microbial changes were investigated using culture-dependent and culture-independent technologies. Specific microbes that discriminate the pre- and post-transportation groups in each evaluated stage were identified through the linear discriminant analysis effect size (LefSe). Principal Co-ordinate Analysis (PCA) on the microbiome profiles showed the distant clustering of the pre-and post-transportation samples in each analyzed stage, demonstrating the significant microbial changes that occur post-transportation.

Further, irrespective of the life stages of fish the transportation significantly lowered the bacterial counts. The major dysbiotic events in the whole larval microbiome included the significant reduction in the microbial abundance and *Actinobacteria.* The reduction in *Actinobacteria* susceptibility of the fish larvae which contains several promising probiotic strains for aquaculture, can be a cause for the increased post-transportation disease. In addition, a significant increase in bacteria that reported by lower larval survival, *viz*. *Vibrio* spp., *Arcobacter* spp. and *Acinetobacter* spp. Further, the genera with presumed beneficial effects, like *Pseudomonas* spp. and marine actinobacterial lineage PeM15 decreased. The changes noted that changes at the phyla level, *viz*. significant reduction in *Actinobacteria* and increased ratio of *Firmicutes* to *Bacteroidetes* can be explored as a stress biomarker for fish larvae, in future research.

The major changes in the juvenile fishes gut microbiome included a significant reduction in the abundance and diversity measures (Simpson and Shannon index of taxonomic metagenomics). There was a significant reduction in *Alphaproteobacteria* and a significant increase in *Gammaproteobacteria*, especially those having opportunistic fish pathogen potential like *Serratia* spp., *Enterobacter* spp., an unidentified genus in *Flavobacteriaceae, Pseudoalteromonas* spp., *Alteromonas* spp., and *Enterovibrio* spp. The significant reduction in *Acinetobacter* spp. and *Empedobacter* spp. was noteworthy observation. The observed reduction in the diversity measures may explain the increased disease susceptibility of the juvenile fish post-transportation.

Conclusions

Loss of microbial diversity and increase in the opportunistic pathogens can be a cause for the increased disease susceptibility of juvenile fish post-transportation.

Increased relative abundance of γ-*Proteobacteria* in the fish gut can be explored as a stress signature in future research.

No changes were observed in the microbial phyla in the guts of fish juveniles whereas several significant changes were noted in the larval microbiome. This might be a reason for higher disease resistance in the juveniles than larvae, following transportation.

Future research for exploring the specific microbial signatures (*Pseudomonas* spp. and marine actinobacterial lineage PeM15 for larvae and *Acinetobacter* spp. and *Empedobacter* spp. for juveniles) as potential probiotics to mitigate transportation stress in marine fish is highlighted.

Management measures aiming to increase the population of the favourable bacteria and suppress the opportunistic pathogens during live transportation are warranted.

Science Corner

An integrative taxonomic approach to the systematics of the genus *Pampus*

In capture fisheries management, fish populations play a critical role in deciding management units employed. Projects like FishPopTrace have been employed in Europe, facilitating use of genetic data and morphological markers, including otoliths for this purpose. Multispecies, multi-gear fisheries landing closely resembling species mixes found in a common ecosystem or fishing ground is widespread in the Indian EEZ. Pomfrets are a low -volume, high value marine fishery in India and an important targeted fishery resource in the Indian Ocean region. However, the taxonomy of the genus *Pampus* has remained complex till date, with literature indicating several cryptic species leading to misidentification and nomenclature issues. This has serious ramifications for traceability concerns in seafood trade and supply chains as well to address sustainability concerns while preparing species specific fisheries management plans. A recent study involving an integrative taxonomic approach with conventional taxonomy tools as well as genetic data and otolith structure, has helped to clarify the taxonomic status of the genus *Pampus* in the Indian EEZ and the new findings with a global outlook is summarized below.

[1]**Roul, S. K., Jeena, N. S., Rajan Kumar, Vinoth Kumar, R., Shikha Rahangdale, Rahuman, S., Ghosh, S.,Rohit, P. and Gopalakrishnan, A.2021. Postulating the modality of integrative taxonomy in describing the cryptic congener *Pampus griseus* (Cuvier) and systematics of the genus *Pampus* (Perciformes: Stromateidae).** | *Frontiers in Marine Science*, 8: 778422. doi:10.3389/fmars.2021.778422

The genus *Pampus* (Family: Stromateidae) commonly known as pomfret, is widespread in the Indo-West Pacific. They are considered as one of the most valuable and popular tablefish that form commercial targeted fisheries in its native ranges including India. The taxonomy of this genus has been uncertain as the similarity in external morphological traits among pomfrets poses problems in correct identification, leading to much controversy regarding species classification, nomenclature, and numerous erroneous GenBank records. Most studies have focused either on either their physical diagnostic characteristics or their genetics, and a review compiling these features was not available.

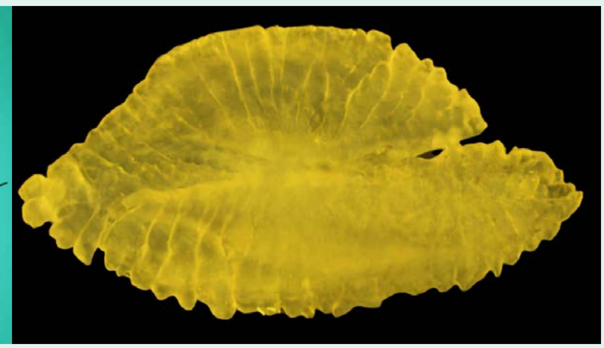
Species forms the bedrock of biology, and is defined by a scientific name that includes its genus and species names. The genus defines the category of the organism at the lowest level. The latest global reports indicate the presence of the following seven valid species in the genus *Pampus* viz. *Pampus argenteus*, *P. minor*, *P. punctatissimus*, *P. chinensis*, *P. cinereus*, *P. candidus,* and *Pampus* sp. Previous genetic studies on pomfrets from India indicated the presence of two putative species viz. *P. candidus* from the Arabian Sea and another cryptic species *Pampus* sp. from the Bay of Bengal (BOB) and confirmed that *P. candidus* so far treated as *P. argenteus* in India is the most common species in Indian waters. *Pampus argenteus* is endemic to the western Pacific Oceans and is entirely absent along the Indian coastline (Divya *et al*., 2019).

Recently, a study by team from ICAR-Central Marine Fisheries Research Institute (CMFRI) has led to new informations regarding the taxonomy of this genus by using an integrative taxonomic approach that combines morphology and genetics of ample number of fresh pomfrets of available

Pampus griseus

This species has so far been erroneously ascribed as *Pampus argenteus.* Adults (>167 mm Standard Length SL) of *Pampus griseus* move in large schools and inhabit shallow waters of diverse bottoms (sandy, silty, rocky and turbid). Juveniles (~ 140 mm SL) are found in depth gradients of 3-30 m and adults mostly in 30-70 m depths. It has restricted distribution in the Bay of Bengal (East Coast of India, Bangladesh, and Myanmar) and Southeast Asia (Thailand, Vietnam, and Malaysia). The proposed English name Bengal Silver Pomfret was given because its type locality was Bay of Bengal, and its forms the major component of pomfret fishery in the region.

Pampus griseus



Otolith of *Pampus griseus*

species (*P. candidus, P. chinensis*, *Pampus* sp.) from various locations along the east coast (Bay of Bengal) and the west coast (Arabian Sea) sampled between 2019 to 2020. Along with these specimens from the Marine Biodiversity Referral Museum of ICAR-CMFRI and digital images of *Pampus* from the Museum National D'histoire Naturelle were also examined. Since traditional taxonomic tools cannot provide a stand-alone platform to solve the taxonomic perplexity of pomfrets, an integrative taxonomic approach was adopted here. The study aimed to establish the identity of the cryptic congener in the Bay of Bengal, re-evaluation of *Pampus* spp. of the Indo-West Pacific for phylogenetic resolution, revision of the systematics and preparation of field identification key for all seven valid species in the genus Pampus. Here, traditional taxonomic tools *viz.* Morphology, multivariate analysis, gill raker shape, sagittal otolith morphology, vertebral count, morphology of transverse occipital canal of the lateral line, and fishery information were integrated with molecular data for all the species fished along the Indian coast, and compared with global literature and database for inference.

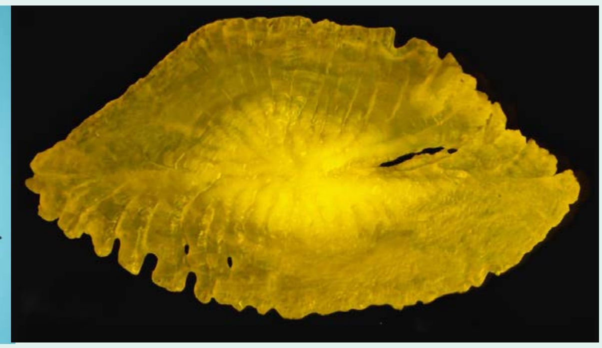
In this study, the cryptic and valid species *Stromateus griseus* Cuvier and Valenciennes, 1833 is resurrected from the synonymy and redescribed as *Pampus griseus* based on specimens from the Bay of Bengal, confirmed by molecular analyses that indicated a limited distribution of the species to the region. Phylogeny of the genus was reconstructed, integrating COI barcodes and concatenated mitochondrial gene sequence data (1822 nucleotides) generated from the available species. The phylograms reconstructed on the concatenated data produced a highly supported clade for the *P. cinereus* complex (*P. griseus*, *P. cinereus*, and *P. candidus*) that share similar features. The clustering of the three sister species indicated a convergent evolution characterized by the elongation of dorsal, caudal and anal fin, a common character of the three (Yin *et al.,* 2019). The tree topology discriminated each species into separate clades with no common haplotypes. There were also two sister lineages i. e., Indian and Pacific Ocean lineages in *P. chinensis* that may be due to the climate-related vicariant events during glacial epochs and the effect of the Indo-Pacific Barrier.

Multivariate analysis has been widely used in species identification and discrimination. The multivariate analysis isolated the *P. griseus* from its counterparts. The morphometric variable like caudal peduncle length (CPL), pre-dorsal distance (PDD), pectoral fin length (PFL), and eye diameter (ED) were important

Pampus candidus

The proposed english name is Indian Silver Pomfret. One of the predominant species in the Indian Ocean it was originally described as *Stromateus candidus* Cuvier and Valenciennes (1833) from Indian waters, and later synonymized with *Pampus argenteus*. It was redescribed as a valid species by Divya *et al.* (2019) based on fish samples from Arabian Sea and Bay of Bengal, and a lectotype was designated, as type specimens were absent.

Pampus candidus



Otolith of *Pampus candidus*

morphometric measurements capable of separating *P. candidus* and *P. griseus*. Marginal overlap was observed in the two groups which suggested their cryptic nature that led to its misidentification as single species (*P. candidus*) till date.

Otoliths are depicted to have high morphological variability with particular characteristics across species and genera and at times considered as species-specific characteristics in taxonomy .Comparison of sagittal otolith morphology of seven valid species of *Pampus* including *P. griseus*, *P. candidus* and *P. chinensis* from Indian waters and four species from Chinese waters revealed that the overall gross morphology of sagittal otolith of *P. griseus* is more similar to *P. candidus*, *P. cinereus, P. chinensis*, and *P. punctatissimus* than others. This character has lesser taxonomic utility to differentiate between closely resembling species, when used alone. The study also suggested that the long and thin fins should not be considered when identifying the species as it gets damaged easily from fishing operations.

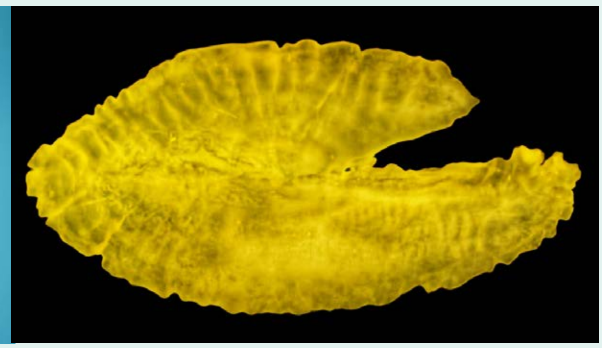
The present study covered the entire Indian coastline and suggested that the three species that are sympatric in the Indian Ocean vary in range of distribution and abundance; *P. chinensis* is more widespread compared to *P. candidus* found in the Arabian Sea and certain areas of the BOB, while *P. griseus* is exclusively found in in the BOB. It is interesting to note that although both species occur in Tamil Nadu, only *P. griseus* has been traced from the fishing ports of the type locality and adjacent regions. The study adds identification markers or diagnostic features (*viz*. re-description) of the pomfret *Pampus griseus* and proposed a new English name 'Bengal Silver Pomfret' due to its restricted geographical distribution in the BOB. The genetic data of the *Pampus* spp. from the GenBank (a database containing diagnostic DNA sequences of all organisms) was also corrected. The authors also suggest some new English names for certain species such as 'Indian Silver Pomfret' for *Pampus candidus* as it is the dominant pomfret species in the Indian Ocean, and 'Japanese Silver Pomfret' for *Pampus punctatissimus* given its original description comes from the Japanese waters.

Collection of species-specific information is vital for long-term sustainable fishery management plans and inaccurate identification of fish species or use of ambiguous names in landing reports will lead to undesired consequences. Precise information on species forms the basis for international trade,

Pampus chinensis

A commercially important species, the stock of *P. chinensis* in the Arabian Sea (Indian Ocean) is forming a distinct lineage from the Pacific Ocean. The species is distributed in the Arabian Sea, Bay of Bengal, East and South China Sea and coastal areas of Malaysia in Southeast Asia.

Pampus chinensis



Otolith of *Pampus chinensis*

consumer safety, biodiversity research and prevention of fraudulence. *Pampus candidus* misidentified as *P. argenteus*, formed a regular export commodity from various ports in India. Present study affirmed that *Pampus argenteus* is completely absent in the Indian Ocean and primarily distributed in the Western Pacific only. DNA-based approaches can be successfully applied as an alternative tool for seafood authentication, even on partially or fully processed fishes when important morphological characters are lost. The authors have summarized the diagnostic characteristics, reviewed the systematics of genus and provided an easy field identification key based on a combination of features. This study has opened various doors to studies related to pomfrets[1]. Further, studies like this can have potential impact on the management of the highly valuable pomfret fisheries, addressing associated seafood trade and traceability concerns in export markets as the species can be identified with complete accuracy.

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