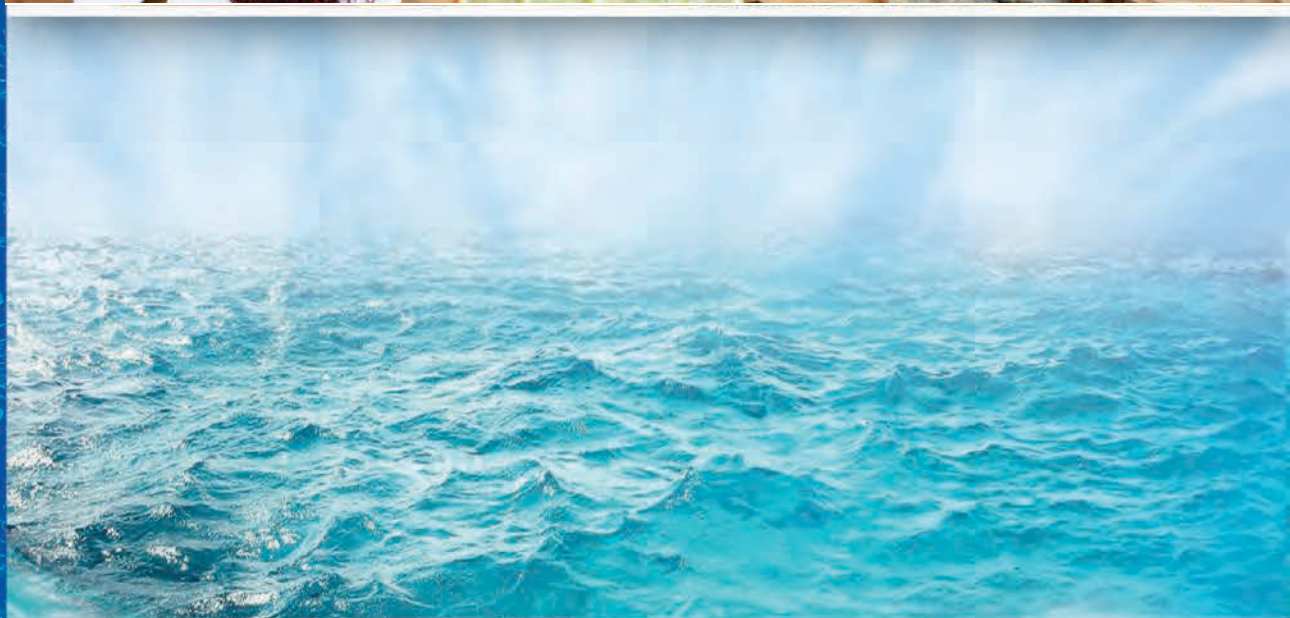




# FISHTECH

## REPORTER

VOL. 07 (2) JULY - DECEMBER 2021



**भा कृ अनु प - केंद्रीय मात्स्यिकी प्रौद्योगिकी संस्थान**  
**ICAR - CENTRAL INSTITUTE OF FISHERIES TECHNOLOGY**

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## FROM THE EDITOR'S BENCH

With increasing prospects in food processing and supply chain infrastructure, the fish processing sector is going for a major leap in future. The allocation from the Union Govt for “PM Gati Shakti Plan” attracts the food sector in a big way. This calls for technologies in all the areas of fish value chain starting from harvesting, post-harvesting to distribution, protecting the commodity from loss of value, both economic and health point of view. Any small technology developed shall be available in public domain which help the interested stakeholder to take up and prosper. The technologies, infrastructure and concepts developed need to be disseminated to the appropriate stakeholders for value realization.

This issue of FISHTECH Reporter discusses concepts, technologies and major research findings from ICAR-CIFT, in the important areas of fish utilization. In the harvest side there are articles discussing on innovative baited gillnets, light assisted squid fishery, targeting trawls for jelly fishes, cage culture - Chinese dipnet combination for better marketing, besides a report on ghost fishing. Improvements in dried fish, flavor separation and encapsulation, seaweed-based value addition and technique to stop melanosis in shrimps provide interest to the readers in the post-harvest side. A couple of articles discuss Solar powered automatic fish feed dispenser providing interest among the aquaculture people. There are also articles discussing on food safety issues, converting waste to feed and few articles on the basic science aspects, particularly on antimicrobial resistance and new approaches to AMR mitigation.

I am sure the articles will prompt interest in the minds of the readers and at the same time enthrust others to write on the half yearly science magazine.







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# A targeted trawl fishery for jellyfish off-Kollam

Chinnadurai S.\*, Gautham K., Paras Nath Jha, Renjith R. K.  
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Jellyfish species are free-moving planktons that drift with ocean currents and appear in irregular quantities all along the coastal waters (Baliarsingh et al., 2020). Some Rhizostome jellyfish have been exploited for more than a thousand years ago which constitute important food in many Asian countries (Omori and Nakano, 2001). Chinese and Japanese are the leading consumers of jellyfish (Kitamura and Omori, 2010), while commercial harvest from the wild commenced in the 1970s with an estimated catch of 500,000 metric tonnes per year. The global landings of edible jellyfish catch was estimated to be more than one million tonnes per year (Brotz et al., 2016) and China alone produced 280,000 tonnes of edible jellyfish in the year 2016 (Leone et al., 2019). In India, the jellyfish as food is still absent due to lack of tradition for edible jellyfish and there was no jellyfish fishery until early 1980s. The jellyfish fishery, processing and export was first reported in India by Chidambaram (1984) from Pondicherry and Tamil Nadu. In the year 1984, about 21 tonnes of jellyfish were processed and exported to Japan, Thailand and Hongkong. However, large scale exploitation of jellyfish started in 2003 mainly due to international market demands and decline of resources from Japanese waters. In the year 2016, about 70 tonnes of dried, salted and frozen jellyfish was exported to Thailand and Vietnam from India (Seair, 2016). Despite the long history of jellyfish exploitation from east coast of India, the information on jellyfish fisheries along the west coast of India is limited. The jellyfish *Crambionella* spp., commonly known as 'karupatti sori' in Malayalam, is one the most abundantly found jellyfish on the Indian coast (Behera et al., 2020).

*Crambionella orsini* was first reported by Nair (1954), who also observed the abundance only for few days in the south-west coast of India. Billett et al. (2006) correlated the high abundance with hypoxic coastal waters of Oman. During the study period, an estimated 2500 tonnes of *C. orsini* were landed. Extensive blooms of jellyfish (*C. orsini*) have appeared in the month of December 2020 in the southwest coast of India. The jelly bloom lasted up to late January 2021. Generally the bloom of jellyfish was considered a menace to coastal fisheries as it caused damages to fishing nets and hindered the fishing operations along the southwest coast of India (Sandhya et al., 2020). However, the species *C. orsini* is emerging as a targeted fishery resource along the coast of Kerala as it fetches reasonable price and due to the increased demand of processed oral arms of jellyfish in south-east Asian countries. Interviews with fishers of Sakthikulangara fisheries harbour during the period from December 2020 and January 2021 indicated that *C. orsini* was the subject of a targeted jellyfish fishery ten years ago-but fishery was sustained for only three consecutive years before gradually ceasing. *C. orsini* bloomed exactly ten years later. The morphometric measurements on 50 undamaged jellyfish (Fig. 2) showed that the average weight of was about 550 g with about 213 g for the oral arm. The jellyfish's umbrella diameter ranged from 16.5 to 21.8 cm, with an average oral arm length of 6.5 cm. The oral arms produced a percentage yield of between 35% and 41%.

Jellyfish are primarily targeted by trawlers on single-day fishing trips and were operated in water depths ranging from 8 to 20 m. On average, 40-50 trawlers land jellyfish weighing between 40 and 50

tonnes per day and the catch was brought by simply loading them onto the deck. Fishermen sell whole jellyfish for Rupees five per kg. Additionally, fishermen stripped off the jellyfish umbrellas while at sea and sell the unprocessed oral arms for Rs.15/kg to processors from Andhra Pradesh. At the landing centre, the umbrellas of all landed jellyfish were removed and only the oral arms were retained for further processing. Approximately 40-50 workers are involved in the process (Fig. 1). The wages for

removing the oral arms were Rs. 1/kg. Unprocessed oral arms were transported in trucks to processing plant (Fig. 2) and after processing it has been transported to Andhra Pradesh, from where they were exported to China. There are only a few studies on the fishery and interannual variation of jellyfish in Indian waters. Additional research on the fluctuation of the fishery and the influence of seawater hydrological parameters is necessary to understand the sustainability of the jellyfish fishery off India's west coast.

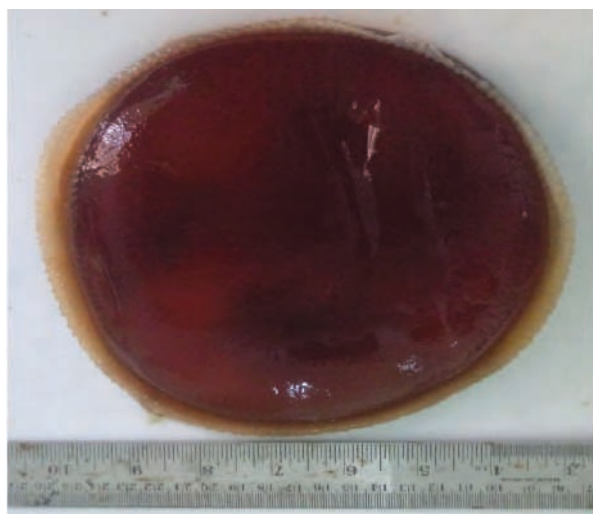
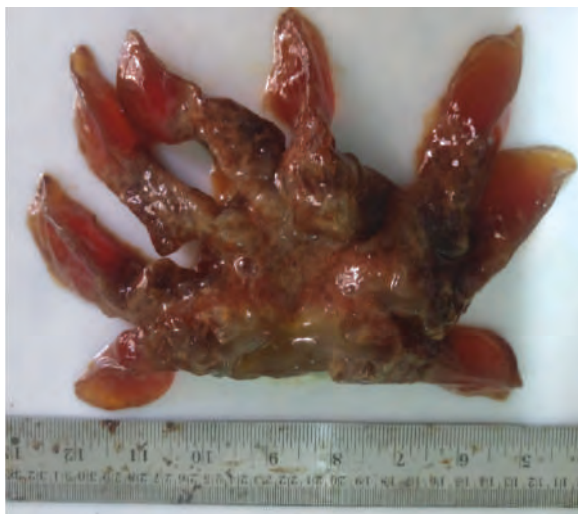
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**A****B**

**Fig.1** Jellyfish *Crambionella orsini* unloaded from trawler (A&B), workers separating oral arms from jellyfish and discarded jellyfish umbrella .

**A****B****C****D**

**Fig.2** Morphometric measurement of *C.orsini* (A&B) and loading of orals arms to the truck for further process (C&D).

## Chinese dipnet-cum-cage culture in Cochin backwaters

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Cochin backwaters and adjoining coastal water bodies in Kerala are one of the richest areas of fish production. Thousands of people around the water body find their livelihood from fishing in the lake. Several types of fishing gears are in operation along the lake targeting all kinds of fishes including juveniles. Among the fishing gears operated in these backwaters, Chinese dipnet, commonly known as 'Cheenavala' is a popular one owing to its structure. Chinese nets operated in the backwaters have reached its present stage of development through gradual evolution from a simpler contrivance for sustenance fishing to the one which is operated on a commercial scale. This gradual development has been attained through the ingenuity of the fishermen who strive to achieve better efficiency, based on their practical knowledge of the gear and the fishery.

Considering the commercial significance of this gear and the magnitude of fishermen engaged for their livelihood, a preliminary study has been conducted

for documenting the developments in Chinese dipnets, changes in fishing operations and associated issues. Study was carried out in five centres namely Kuzhupilly, Kadamakudi, Ezhikkara, Cheranellur and Koonammavu in Ernakulam district during September 2020.

Major structural modifications noticed were replacement of wooden poles of the gear with GI pipes as it is easily available, having better durability and withstand strong winds and currents. Dipnets with 22mm mesh size are operated for fishes and 16mm mesh size for shrimps. Refurbished bike engine with pulley system were used for lifting the nets which were done manually earlier days, is another noticeable change in a few units. Use of white or yellow coloured LED lights (9 -90w) instead of kerosene lamps for attracting fishes is very common now. Similarly raw or fried cattle feed is used as attractant by some fishermen to increase the catch.



*Dip net night fishing using light in Cochin back water*



Currently the fishery is facing a lot of problems due to various reasons. Floating aquatic weeds chocking fishing nets is a severe issue in Cochin backwaters. As per recent reports few dipnets along with supporting structure and platform were almost fully damaged due to huge quantity of floating weed drifted over the net at Fort Kochi. Reducing depth of water bodies due to heavy siltation is another challenge faced by fishers operating in shallow regions of backwater. Proliferation of jellyfish in the lake during the months from June to September or even beyond affect the fishing operations and fishers stop fishing during this period.

Juvenile catch is a concern in dipnet fishery due to the use of small mesh webbing. To overcome the issue the *Cheenavala* fishers had started a new method of capture-based cage culture system. Small sized high values fishes captured in dipnets were transferred for culturing in cages installed below or adjacent to the platform of the dipnet. *Caranx* sp. (*Vatta*), *Lutjanus* sp. (*Chempalli*), *Etroplus suratensis* (*Karimeen*) and other commercially important fishes were mainly stocked in the cages. One to three cages were erected nearer/below a *cheenavala* platform. Fishes transferred into the cage for fattening were fed with trash fishes/shrimps and during off season slaughter house waste were also used as feed.

Two walled cages with 3.5m x 3.5m x 1.5m in size are seen in one of the units selected for the study. Outer cage is made of HDPE webbing of 20 mm mesh size with 6 mm twine size (cage size is 3.5m x 3.5m x 2.0 m) and inner cage with PA webbing of 12 mm mesh size with 3 mm twine size (size is 3.0m x 3.0m x 1.5 m). Usually, *chempalli* and *karimeen* were kept in between the inner and outer cages and other high value fishes were kept in the inner cage, which helps to minimize the algal growth in the outer and inner cage walls and allow free flow of water.

Fishes stocked in the cages were shown better growth rate and cages require minimum or no maintenance. As seed and feed are available free of cost, at present fish fattening is popular among the *cheenavala* fishers.

Though catching fishes below minimum legal size is not recommended, growing juveniles caught in dipnets to marketable size will improve the economics of Chinese dipnet fishermen, who are facing lot of problems to earn their livelihood. More cages may be erected in the vicinity of stationery gears like Chinese dipnets and hatchery produced seeds may be stocked in the cages to make the venture more economical.



*A cage installed below the platform of Chinese dip net*

## “Light on” Light assisted seasonal fishery of squid along Saurashtra coast of Gujarat

**Prajith K. K.\*<sup>1</sup>, Parmar Ejaz A. Rahim<sup>2</sup> and Anand Narayanan D.<sup>2</sup>**

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In India, artisanal fishery plays a pivotal role in the livelihood of the fishermen and contributes 81% of the sector. The small-scale fishermen carry out fishing with traditional craft and gears with or without outboard engines (Kanthiah, 2010). The operation is mainly confined to the near shore region with relatively small crafts and simple fishing gears. In small-scale fishing, fishers mainly rely on the experience and traditional ecological knowledge and uses fishing practices known for centuries (Jadhav, 2018). One of the major problems faced by the small-scale fishing sector of the world is competition from the mechanized sectors and declining catches which pressure them to make technological changes. Gujarat is one of the leading states in marine fish production and currently holds the second position in the total marine landings in India. Gir-Somnath ranks first in total marine landings in the state and second all over the country. Gujarat fisheries is dominated by trawlers followed by gillnet setters. Doll netters, artisanal long liners, bag net setters are some of the other fishing gears operated in the state. About 11.2% of total marine captures is contributed by the small-scale fisheries of the state. (CMFRI, 2019).

Light and visual systems highly influence the interaction of marine animals with fishing systems (Arimoto et al., 2010). The light assisted fishing has been practiced by humans from historic time and it has been started as a simple approach like burning fire on seashore to attract fish. Later this technique become part of common fishing method which promises successful and effective harvest of commercially important species. Many Asian countries viz, Japan, Korea, Malaysia, Vietnam, Thailand, and Philippines widely practiced light fishing. In Japan, all the squid jigging boats, stick held dipnets and more than fifty percentage of the fleet operating purse seines use light for fishing (Mohamed, 2016). In India, with a history of more than 200 years, the Chinese dipnets of Kerala, use lights to attract fish

over the dipnet. But the use of artificial lights in the commercial fishery of India has reported from the west coast of the country in the end of 2013. The order issued by the department of Animal Husbandry, Dairying & Fisheries (DADF), Govt of India dated 10<sup>th</sup> November 2017, prohibits the installation and use of artificial lights in mechanized and motorized trawlers, purse seines and gill net setters in the Indian Exclusive Economic Zone.

In order to study the coastal light assisted fishery of Saurashtra coast, a survey was conducted along the Veraval and Jaleswar region of Gujarat. The study revealed that almost 10-15 years back, there was a targeted light assisted fishery for shrimp with cast net. During those days battery operated torch and burning fire were used as light source. Later on, during night time (multiday fishing) fishers observed the attractive response and schooling behavior of squid to the light attached with gillnet setters. Understanding the behavioral response of these economically important molluscs, few fishers started using artificial lights targeting squids. Gradually the practice became more popular and advanced and gradually spread throughout Saurashtra coast and presently at Gir Somnath district alone more than 200 boats are engaged in light assisted targeted fishery of squid.

In Veraval, gill nets of various mesh sizes are operated throughout the season. Depending on the season, catch from the gillnets may fluctuate. Once the catch from gill net is less, the fishers shift to the light-based fishing for squids. In Saurashtra coast, light assisted fishing is purely seasonal. Fishing starts in the middle of October with peak landing in December and January (winter months). Fishing is carried out in very near coastal waters and boats can be easily spotted from the beach itself. Fishing is restricted within 50m depth. Based on the availability and detection of school, cast nets are be operated from evening to morning. Besides cast net, scoop net is also



used for harvesting the aggregated cephalopods. As squids are columnar, they constitute major catch. While bottom dwellers like cuttlefishes, other pelagic and demersal fin fishes became bycatch. The average catch ranges from 20 - 500 kg in a single trip. Entire unit is assisted and operated by 2-3 fishermen. The same FRP outboard motor boats (OBM) used for gillnet fishing is used for light fishing with suitable modifications. Dimension details of the vessel are given in Table 1

**Table : 1** Details of FRP boats used for light assisted fishing

Specification	LoA (m)	Breadth (m)	Depth (m)	Engine Power (hp)
<b>Dimension</b>	5-12	0.5-2.5	0.5-1.5	6-9.9

#### Specification of light used

LED bulbs are the main source of light. Some vessels are equipped with halogen bulbs and fox lights also. A typical fishing unit consists of 80-200 bulbs arranged in a specially fabricated frame. Each frame is with three panels contains 10 bulbs (10X3=30 bulbs). There will be 4-6 such detachable frames in a single boat. Besides 10X3 bulb combination, 10X4, 6X3, 12X2, 7X4 panels are also common. LED bulbs (9W) of various brands, are used in the panel. Each panel consist of 270-360W and entire fishing unit is having a power of 1000-1500W. A single bulb cost around 70-120 INR. Generators are used for power supply and illumination of bulb. Besides Jaleshwar, Dhamlej, Hirakot and Rupen are the major light fishing locations of the Saurashtra coast. In Rupen bandar of Devbhoomi Dwarka district, we observed big unit of light fishing boat fitted with 2340 W lights (Fig.3).

As the light assisted fishing is seasonal, additional investment is required for purchasing bulb, illuminating energy source, and fishing gear (cast net, scoop net) Table 2. The same fleet used for gillnetting are used in light fishing also. The operational cost is 1000-4000 INR per day and the average catch ranged from 20-500kg/trip. The profit depends on the catch rate and ranges from 2000-15,000 INR per trip. As the

fishing is mainly conducted in the near shore water, fuel required for this fishing is comparatively less. The total profit is divided into two parts and 40-60% goes to fishermen which then divide among themselves and the remaining goes to the boat owner. Some part of the profit is used for the maintenance of vessel.

In India, squid rank next to shrimp as the most crucial seafood for export. Cephalopods are actively fished in the artisanal fishery with highly selective gears and fishing methods (Reid et al., 2005). Hooks, jigs, trawls, various types of seines, traps, dol nets, and spears are the gears used for the exploitation of cephalopods in Indian waters (Rao, 1954., Lazarus and Achil, 1984, Nair, 1985, Silas, 1985, Joel and Ebenezer., 1987, Rao, 1996, Sasikumar et al., 2006., Venkatesan and Shanmugavel, 2008., Sundaram & Sawant, 2013, Muniyapillai et al., 2016). There are very few records on the use of cast net and scoop net

**Table : 2** Details of additional investment required for setting up a typical light fishing unit.

	Unit cost	Total
Generator	15000	15000
Cost of bulb	70	8400
Bulb holder	30	3600
Frame fabrication charge	1000	4000
Wire, switch, etc.	-	500
Cast net	2000	6000
Scoop net	200	400
TOTAL (INR)	-	37900

for harvesting squids. In 1978, there was a report on the mass harvest of male squids using cast net and scoop nets along the Alleppy coast of Kerala (Meiyappan and Mohamed, 2003). Cast nets are the simple, oldest, and widely operated fishing gear both in marine and inland sector for harvesting the fishes inhabits the shallow water or with schooling behavior. The fishing is confined to specific area where the net is operated. There are reports of light assisted fishery of squids and cuttlefish with other fishing gears. The major advantages of light assisted squid fishing of Saurashtra coast are, the fishery promises comparatively less fishing effort with higher catch. As the fishing is seasonal nature, there is an overall

reduction in fishing efforts. Only cast net and scoop nets are used in the fishery which ensure fishing in a responsible way.

Spawning of squids is observed throw-out the year and coastal aggregation of large number of male individuals are reported along the south west coast and vulnerable for exploitation (Meiyappan and Mohamed, 2003). The females may move to the deeper water for egg laying. In the present study, only technological aspects of light assisted squid fishing is discussed. More investigation and a full seasonal study on the catch composition and biology of the catch is suggested to get full information and impact of light fishing.



*Fig.1 View of a typical light assisted fishing unit of Jaleshwar, Veraval, Gujarat*



*Fig.2 Fitting light panels to the vessels.*



*Fig.3 Larger light fishing units of Rupen bandar, Devbhoomi Dwaraka, Gujarat*



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## Baiting of gillnets: An innovative approach to increase catch efficacy

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Gillnets are one of the most commonly used fishing gears worldwide and frequently regarded as highly selective. Success of gillnet operation is dependent on the activity of fish in the vicinity of the gear, which increases the likelihood of physical contact. Stimuli, those appeal primarily to the olfactory system, are widely used in passive fishing operations such as traps and longlines, as fishes being seen at close proximity to the fishing gear. The success of bait-dependent fishing is highly dependent on knowledge of the targeted fish's feeding behaviour and an understanding of olfactory efficacy, or the ability to detect and locate the source of olfactory plumes efficiently (Hossucu, 1991). Various researchers have asserted that increasing the likelihood of a gillnet encounter is possible by luring the fish to the nets (Engas et al., 2000; Kallayil et al., 2003; Ozdemir and Erdem, 2006).

In this context, and with the goal of increasing the catch efficiency of gillnets, a preliminary work was attempted by baiting marine gillnets. Experiments were conducted off Cochin in Eastern Arabian sea during 2018 to 2019 at a depth range of 80-100 m. Experimental gillnets were made of polyamide multifilament (210x9x3) having a mesh size of 140 mm, and rigged at a hanging coefficient of 0.5. Due to its widespread availability and strong odour, Indian mackerel (*Rastrelliger kanagurta*) was chosen as bait. The chopped fishes were placed in small-mesh pouches and tied to each 10 g of head rope of the experimental nets measuring 1000 metres in length and 7.5 metres in vertical height (Figure 1). Control net (1000 m) was of identical specification as of the experimental net devoid bait pouch. Nets were set at dawn following a soaking period of four to five hours, and hauled at midnight.

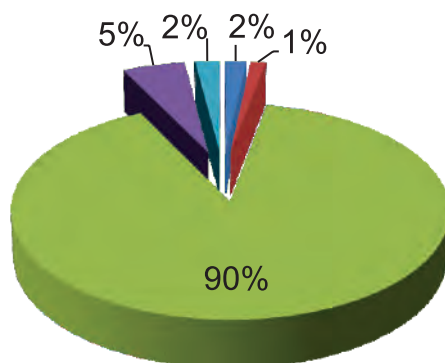
The catch per unit effort per soaking time of combined experimental gillnet was calculated as 5.08 kg/h. Baited gillnet landed more catch (93.8%) than non-baited gillnet (6.2%) (Figure 2). In the baited gillnet, the catch primarily comprised of *Manta birostris*, *Scomberomorus commersoni*, *Lobotes surinamensis*, *Megalops sp.*, *Cyprinoides* and *Katsuwonus pelamis*. where as in non-baited gillnet, *Katsuwonus pelamis*, *Rastrelliger kanagurta*, *Cypselurus sp.* and Leather jack contributed the catch (Figure 3 & 4). This indicated that carnivorous/top predatory fishes, which frequently fetched a higher price than the species caught in the control gillnet, interacted more with baited gillnets, indicating the bait efficacy.

Engas et al. (2000) compared catch rate of baited and non-baited fleets of gillnets using mackerel as bait and found significantly higher catch (61%, 23% and 36% for cod, ling and Greenland halibut, respectively) in baited fleets. Similarly, effect of baiting and colour combination on the efficacy of gillnets was studied in Butte Lake, New Mexico, showed increased catches between 80% and 85% (Jester, 1977). Dartay and Duman (2016) also reported higher catch efficacy in baited part of gill net than non-baited part. This study demonstrates that baiting can significantly increase the catch rate of gillnets. Baiting may also significantly reduce soaking time due to the increased CPUE in baited gillnets. A relatively short soaking time would benefit obtaining fresh catch, reducing catch loss due to predator depredation, and minimising gear loss. Understanding the food search behaviour and feeding mechanism of commercially targeted fish is critical for future research aimed at improving the efficacy of gillnet fishing through the use of bait. Extensive research is being conducted to learn more about the bait preferences of various target species.



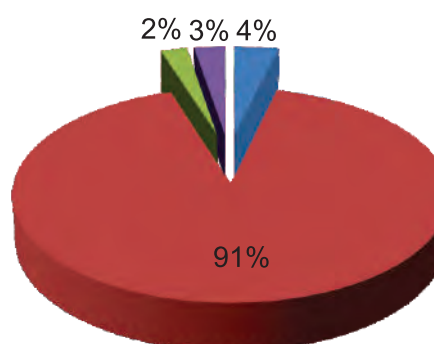
**Fig.1** Experimental gillnet ready for operation

#### Species composition in baited gill net



■ Lobotes surinamensis    ■ Megalops  
 ■ Manta birostris        ■ Scomberomorus commers  
 ■ Katsuwonus pelamis

#### Species composition in non-baited gill net



■ Leather jack        ■ Katsuwonus pelamis  
 ■ Rastrelliger kanagurta    ■ Cypselurus sp.

**Fig. 3 & 4** Major species contributed by baited and non-baited gill net

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## Electron beam irradiation: An alternative method to improve the dried fish product quality

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Fish and fishery products are important source of protein, fat, essential amino acids, minerals, vitamins and other nutrients. Drying is one of the oldest known methods of preservation of food. In India, consumption of dried fishes is about 32% of the total marine landings and about 17% of the total catch used for the production of dry fishes (Sheetal et al., 2017). Bombay duck (*Harpodon nehereus*) is one of the most abundant and preferable marine species in Maharashtra and Gujarat region (Chakrabarti, 2010). *Acetus* sp. form one of the primary dried food items preferred in north west coast of India. It contributes about 20% of marine shrimp landing and constitute seasonal fishery along the Maharashtra coast. *Acetus*, locally called Jawala in Maharashtra, caught as by-catch during trawling is discarded back to sea due to low price. *Acetus* is mostly consumed in dried forms (Jaiswar and Chakraborty, 2005). Electron beam irradiation (EBI) is a non- thermal processing technique gaining more attention by a food processor to improve the shelf-life and preserve its nutrient value. The advantage of the electron beam irradiation over gamma irradiation that it can be applied in a bidirectional manner in which the irradiation can come into contact with the food product from the top and bottom of the sample. This penetration can offer the advantage of a more uniform application of the irradiation, which can lead to a more effective elimination of bacteria, particularly on product surfaces (Lewis et al., 2002)

In the present study, quality of electron beam irradiated dried Bombay duck (*Harpodon nehereus*), dried Jawala shrimp (*Acetus* sp.) were studied. Samples were purchased from local fish market, packed in polyethylene pouches and exposure to electron beam irradiation in a linear EB RF accelerator (EB tech., BRIT, Navi Mumbai). The irradiation process parameters used include Energy 5 MeV; beam power 40 kW; beam current-0-4.5 mA; conveyer velocity of 10m/min; sample thickness 1-3cm. The doses of electron beam used were 0, 2.0 kGy, 4.0 kGy and 6kGy. All the samples were kept at room temperature. Biochemical and microbiological qualities were analyzed.

Proximate composition of dried Bombay duck and *Acetus* sp. are given in table 1. The moisture content is one of the important parameter which determines the quality of dried fishes. In general, the moisture content less than 15% in dried fish is recommended better for longer keeping quality. However, a reduction in moisture content of fresh fish by drying to 25% will stop bacterial growth and reduce autolytic activity. In the present study, Moisture content of dried Bombay duck ranged between 17.01-17.8%. Protein content ranged between 64.2 - 68.33%. Fat content varied from 3.84 - 4.57%. Ash content varied from 15.89 - 16.43%. There is no significant difference in the proximate composition of irradiated and non-irradiated samples.

*Table 1: Proximate composition of electron beam irradiated dried Bombay duck and Acetus sp.*

Sample/Parameter	Moisture (%)	Protein (%)	Fat (%)	Ash (%)
<b>Bombay duck</b>				
Control	17.78±0.25	65.49±0.10	4.57±0.05	16.33±0.10
2.0kGy	17.66±0.20	64.2±0.05	4.28±0.04	16.43±.15
4.0kGy	17.52±0.15	64.51±0.02	4.26±0.10	16.27±0.25
6.0kGy	17.18±0.20	68.33±0.20	3.84±0.15	15.89±0.20
<b>Acetus sp.</b>				
Control	17.77±0.30	66.76±0.10	3.10±0.30	12.80±0.10
2.0kGy	16.71±0.25	66.50±0.20	2.93±0.20	12.64±0.20
4.0kGy	16.92±0.20	65.80±0.08	2.96±0.15	12.69±0.25
6.0kGy	17.18±0.15	66.40±0.06	2.60±0.10	12.60±0.20

Biochemical quality of electron beam irradiated dried Bombay duck and Acetus sp. are given in table 2. In the present study, control had higher TVB-N in both dried Bombay duck (217±1.50mg%) and dried acetos sp. (260±1.40mg%) than irradiated one. Moreover, Peroxide value was higher in irradiated sample (3.02-3.74 milliequi.O<sub>2</sub>/kg) than control in dried Bombay duck. Similar trend also observed for dried acetos sp (control-3.62±0.02milliequi.O<sub>2</sub>/kg; Irradiated- 5.13-7.55milliequi.O<sub>2</sub>/kg). Sinduja et al. (2015) observed

significant reduction in TVB-N and TMA-N irradiated dried barracuda.

Microbial analysis of dried Bombay duck indicated that total bacterial count (3.8-3.3 log cfu/g) was lower in the irradiated sample than control (4.35 log cfu/g). Similar trend also observed for electron beam irradiated dried Acetus sp. (Table 3). Sinduja et al. (2015) observed significant reduction in Total Plate Count (TPC) count in irradiated dried barracuda.

*Table 2: Biochemical quality of electron beam irradiated dried Bombay duck and Acetus sp.*

Sample/Parameter	pH	TVB-N (mg%)	TMA-N (mg%)	PV (milli.eq.O <sub>2</sub> /kg)	TBARS (mg MDA/kg)
<b>Dried Bombay Duck</b>					
Control	6.72±0.05	217±1.50	35±0.65	1.74±0.06	0.58±0.02
2.0kGy	6.57±0.02	196±0.90	30±0.80	3.51±0.15	0.77±0.01
4.0kGy	6.59±0.01	196±1.20	32±1.20	3.74±0.20	0.70±0.04
6.0kGy	6.62±0.02	195±0.85	30±0.80	3.02±0.50	0.79±0.02
<b>Dried Acetus sp.</b>					
Control	7.7±0.01	260±1.40	42±1.20	3.62±0.02	0.59±0.01
2.0kGy	7.87±0.01	238±0.80	35±0.80	5.13±0.05	0.65±0.02
4.0kGy	7.82±0.02	225±2.20	28±0.95	5.40±0.01	0.76±0.02
6.0kGy	7.76±0.01	225±1.80	30±1.25	7.55±0.03	0.85±0.03



**Table3:** Microbial quality of dried Bombay duck and *Acetus* sp.

Sample/Parameter	TPC (log cfu/g)
<b>Bombay duck</b>	
Control	4.35 ±0.10
2.0kGy	3.80±0.05
4.0kGy	3.60±0.02
6.0kGy	3.30±0.06
<b>Acetus sp.</b>	
Control	4.10±0.04
2.0kGy	3.90±0.02
4.0kGy	3.80±0.01
6.0kGy	3.00±0.03

Organoleptic quality of the dried fish products were evaluated in terms of in color, odor, texture, insects' infestation, and overall quality. The color of dried Bombay duck was found to be whitish to yellowish. The color of dried *Acetus* sp. found to be brownish to reddish. Texture was firm and flexible in all samples. No insects' infestation was found in the

dried products. The overall acceptability of the products does not showed any significant difference between the samples. Results from the study suggested that electron beam irradiation can be used as an alternative method to improve the dried fish product quality during storage.

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# Extraction, Encapsulation and Characterization of seafood flavour

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Seafood flavour is one of the high value products and underutilized fish, shrimp and fishery waste serve as the important source for its extraction. Flavour peptides are oligopeptides that differ in order and length of amino acids and contribute to the distinctive taste viz., sour, sweet, bitter, umami and salty (Fadda *et al.*, 2010). Seafood flavours have been extracted from protein hydrolysates of the marine resources, such as fish, shrimp, clam, crab, and seafood by-products as well as seaweed (Laohakunjit *et al.*, 2014; Breternitz *et al.*, 2017). Several studies have been reported on flavour peptides and their utilization in food products such as gluten free pasta and gluten free bread (Vijaykrishnaraj *et al.*, 2015; 2016). Seafood flavour peptides extracted from low value fish and fishery waste and preserved through microencapsulation are an attractive investment that can bring major innovations in food industry. The present study aims to extract flavour peptides from underutilized shrimp and to study the antioxidant and functional properties of extracted flavour.

Low value marine shrimp (*Solenocera* spp.) with a composition of 82.5% moisture, 9.2% protein, 1.6 fat % and 8.8% ash were utilized for flavour extraction.

The seafood flavour was prepared by pepsin and trypsin hydrolysis (at 0.5% of the protein content of the shrimp). Shrimp mince (100 g) was mixed with preheated Glycine-HCl buffer (0.02M, pH 2) and hydrolysis was carried out by addition of 0.5% pepsin (at 37°C for 2 h) followed by trypsin digestion (pH 6.5, 37°C for 2 h). The enzymes were inactivated by heating the enzyme-substrate mixture at 90-95°C for 10 min and the hydrolysate was collected by centrifugation at 3000 rpm for 15 min. The supernatant was dried in microwave vacuum drier (MF) and spray drier (SF) and are collected as seafood flavours. The yield, degree of hydrolysis (%) and protein contents (%) were in the range of 12.16 to 12.75%, 22.19 to 22.5% and 83.13 to 83.56%, respectively. MF showed higher redness ( $a^*$  12.17) and yellowness ( $b^*$  37.18) compared to SF (Fig. 1). The antioxidant indices viz., IC<sub>50</sub> value of DPPH radical scavenging activity and metal reducing power for MF and SF were 0.5mg/ml and 0.69mg/ml, and 0.34 and 0.283, respectively. Results of the study are in line with results reported by Latorres *et al.* (2018), where, hydrolysates from shrimp exhibited the highest antioxidant power for DPPH and reducing power. Emulsifying activity index (EAI) for MF and SF were



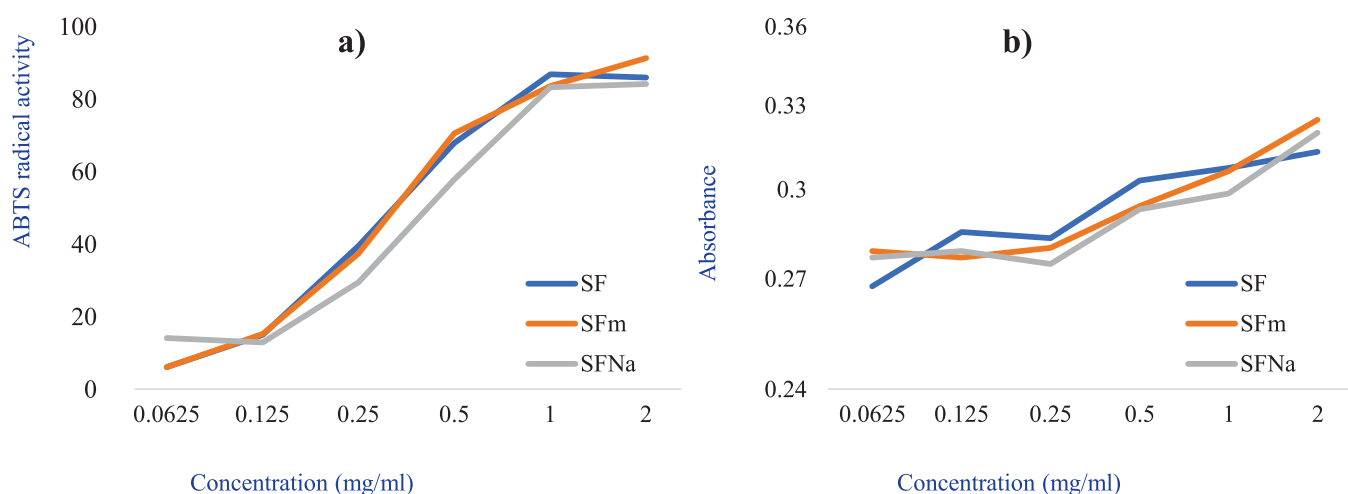
Fig. 1 Dried Seafood flavour- Microwave vacuum dried (MF) and Spray dried (SF)



22.49 m<sup>2</sup>/g of protein and 24 m<sup>2</sup>/g of protein, respectively. Similar results were observed in the study of protein hydrolysates from white shrimp (*Litopenaeus vannamei*) hydrolysed with Alcalase (22.5±0.5 m<sup>2</sup>/g) and Protamex (22.3±0.5 m<sup>2</sup>/g) (Latorres *et al.*, 2018). However, emulsion stability index (ESI) of the hydrolysate reported by Latorres *et al.* (2018) was significantly lower than the results of the present study (ESI for MF and SF were 12.05 min and 15.27 min, respectively). Although having good antioxidant and functional properties, seafood flavor was found to be hygroscopic in nature and to overcome this problem, encapsulation of seafood flavor was performed.

Emulsions were prepared by employing the following combination of core materials, SF<sub>m</sub>: maltodextrin and gum arabic (1% each), and SF<sub>Na</sub>: maltodextrin, gum arabic and sodium caseinate (1% each) with magnetic agitation, until complete dissolution. Then 0.5 ml Tween20 was added to the solution and homogenized at 20000 rpm for 10 min. The spray drying process was performed in a

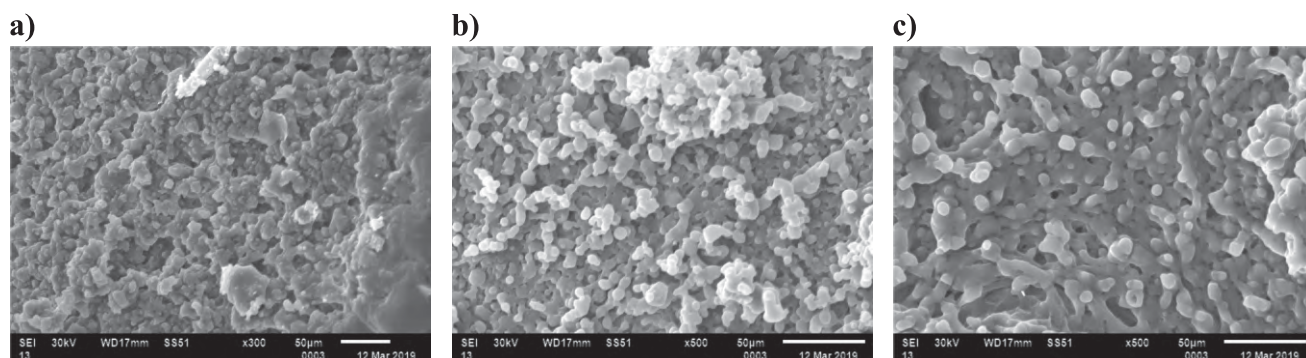
laboratory spray dryer with inlet air temperature 180°C and the outlet air temperature varied from 71 to 72°C. Antioxidant properties (DPPH scavenging radical activity, ABTS radical activity, ferric reducing power assay and metal ion chelating capacity) of non encapsulated seafood flavor (SF) and encapsulated flavor (SF<sub>m</sub> and SF<sub>Na</sub>) were analysed. The encapsulated flavour i.e., SF<sub>m</sub> showed a higher ABTS scavenging activity (91.21 % at 2mg/ml concentration) and reducing power (0.34) compared to the non-encapsulated peptides (SF) and encapsulated SF<sub>Na</sub> (Fig. 2). All the sample exhibited scavenging ability lower than 50% for DPPH radicals and also had very low metal ion chelating property. ABTS scavenging activity of SF<sub>m</sub> and SF<sub>Na</sub> were reduced from 91.21 % to 45.72%, and 84.32% to 44.02%, respectively, at the end of two months storage at ambient temperature while ABTS scavenging activity of non-encapsulated flavour reduced faster from 86.09% to 40.32% after 4 weeks. Furthermore, encapsulated flavours (SF<sub>m</sub> and SF<sub>Na</sub>) are less in hygroscopic in nature as compared to the non encapsulated sample.



**Fig. 2** Antioxidant properties of encapsulated and non encapsulated seafood flavor a) ABTS radical activity and b) Ferric reducing power assay.

Scanning Electron Microscopy (SEM) analysis of the microencapsulated and spray dried seafood flavour showed that the three-dimensional network of pure seafood flavour was less tightly oriented with high porosity and formation of link bridges, due to its

higher hygroscopic nature (Fig. 3a). The electron microscopy images further indicated the presence of particles with different sizes with smooth, wrinkled or spherical surfaces and matrix-type structure (Fig. 3b and 3c)



**Fig. 3** Scanning Electron Microscopy (SEM) images of a)  $SF$ , b)  $SF_m$  and c)  $SF_{Na}$

From the results, it can be concluded that the enzymatic extraction of shrimp flavour followed by spray drying and encapsulation yielded seafood

flavour with higher antioxidant and functional properties that finds application as flavouring agent in innovative seafood flavour rich food products.

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## Effectiveness of beetroot powder, citric acid and EDTA combination for prevention of melanosis in shrimp

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Shrimps are seafood with high delicacy and preferred among consumers. It is comparatively a perishable commodity with shorter shelf life. Melanosis or blackening is an important quality issue affecting crustaceans especially shrimps. It is a biochemical reaction in which polyphenoloxidase (PPO) oxidizes phenols to quinines, further non-enzymic polymerization of quinines lead to dark pigmentation with high molecular weight (Benjakul et al., 2005). These dark pigments called melanin are formed and gather mainly below the carapace of the cephalothorax. Even though, melanosis or black spots are found harmless to consumers, market value and acceptability by consumers will get reduced resulting monetary loss to seafood industry. Treatment using sulfite depends upon the industry in order to prevent melanosis formation for many years. Sodium metabisulfite (SMS), a known allergen can lead to many health issues.

Beetroot is rich in phenolic compounds and water-soluble nitrogenous pigments, called betalains. Beetroot powder was prepared by oven drying method and powdered to fine form. A combination of beetroot powder, citric acid and EDTA was evaluated for its efficacy in reducing melanosis formation in white leg shrimp (*Litopenaeus vannamei*) during iced storage in comparison with control and shrimp treated with 1.25% sodium metabisulphate (SMS). Two treat-

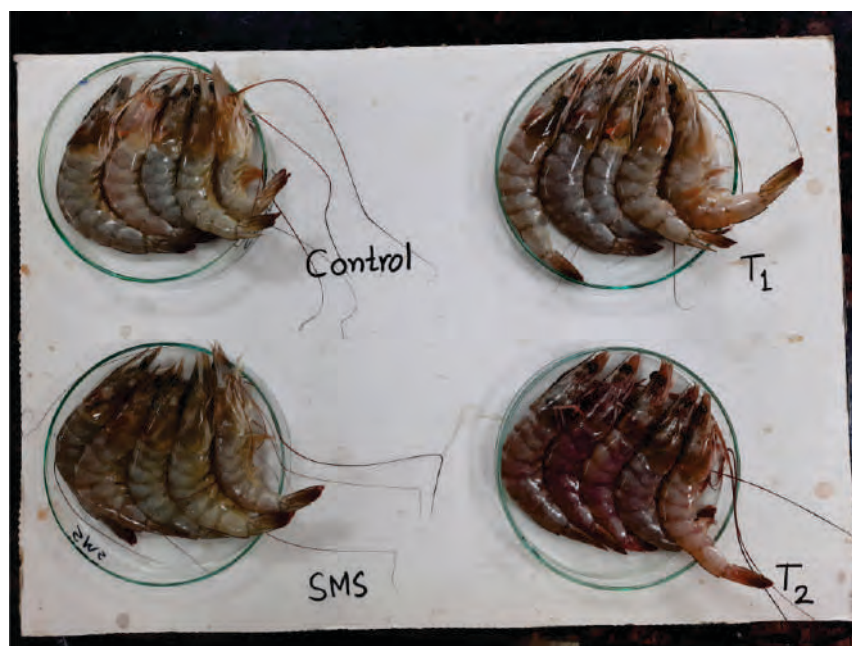
ments were carried out with a combination of beetroot powder at 0.5 and 1% with citric acid at 1% and EDTA at 0.1%, in a ratio of 1:1(w/v) for 15 minutes at 4°C. Treated samples were drained, packed and stored under ice. The changes in melanosis formation, quality and safety were evaluated during storage. Melanosis assessment of white shrimp was carried out through visual inspection using ten-point scoring as per the method of Montero et al. (2001).

pH of control and treatments showed an overall increasing trend. In case of control TVBN reached 30.8 mg% on 9<sup>th</sup> day. T1 (0.5%) and T2 (1%) reached TVBN value of 21 and 18.2 mg% respectively on 9<sup>th</sup> day. Control crossed aerobic plate count of 7 log CFU/g on 7<sup>th</sup> day of storage while T1, T2 and SMS crossed 7 log CFU/g on 9<sup>th</sup> day of storage. Melanosis score of control increased rapidly and reached melanosis score of 6 (Notable, 40 to 60% of shrimp surface affected) on 5<sup>th</sup> day and 8 (severe, 60 to 80% of shrimp surface affected) on 7<sup>th</sup> day. Melanosis score of T2 and SMS was similar till the 5<sup>th</sup> day of storage. Signs of melanosis started appearance in SMS and beetroot extract treated samples from 5<sup>th</sup> day of iced storage onwards. Hence beetroot powder in combination with citric acid and EDTA can be used as an effective alternative to SMS in prevention of melanosis in shrimp.

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*Fig. 1 Melanosis formation on 5<sup>th</sup> day of iced storage*



*Fig. 2 Beetroot powder prepared*



## Database on shrimp processing waste from organized shrimp processing sector in India

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Shrimp is mainly pre-processed as head on shell on (HO), headless shell on (HLSO), headless shell less (HLSL), peeled deveined (PD) and peeled undeveined (PUD) in the organized shrimp processing sector in India. These processes generate huge quantity of shrimp processing waste, which can be further utilized for the development of value-added by-products like chitin and its associated derivatives. The quantity of shrimp processing waste generated was integrated into a database to provide an overview of year-wise and item-wise shrimp processing waste generated. This database developed in Microsoft Office Access (MS Access) and would help the user (students, researchers, academicians and policy makers) to retrieve and analyze the shrimp processing waste generated. The design of database included formulation of tables to store data, queries to search and retrieve data and an administrative panel form to view, add or update the data records in the tables and report generation to analyze and print data in specified formats.

### Design of Tables

A database comprises of one or more tables. Each table stores data as rows and columns. The horizontal entities are called rows and represent a record, for example here each row holds particulars like year-wise shrimp processing waste generated from the processing of items such as headless shell less, headless and shell on, peeled deveined and peeled undeveined and total shrimp waste, all in the unit of tonnes. Similarly, the vertical entities are called columns and they hold a specific type of data such as numeric, dates or text. The data fields are year, HL (Tonnes), HLSO (Tonnes), PD & PUD (Tonnes) and

total shrimp waste (Tonnes) are declared as numbers. Each row is uniquely identified using a primary key. Here, in the data field year is the primary key and it uniquely identifies each record from the shrimp processing waste database table. Table design for shrimp processing waste is given in Figure 1.



Field Name	Field Type	Field Properties
Year	Number	Primary Key
HL (Tons)	Number	
HLSO (Tons)	Number	
PD & PUD (Tons)	Number	
Total Shrimp Waste (Tons)	Number	

Fig. 1 Table created for the database

### Design of Database Queries

A database query finds and retrieves data that meets specific search conditions. It permits the user to search, compile and retrieve data from the database. Queries can also be designed to create, read, update and delete the database tables. Query to retrieve all the data records from the database was created. Similarly, queries are designed to retrieve year-wise data, period-wise data, item-wise, HL – period-wise data, HLSO – period-wise data, PD & PUD – period-wise data and total shrimp waste – period-wise data.

### Design of Database Forms

A user interface form was created in the database application. It helps the user to view records under different category. Figure 2 shows the user panel form. Form controls are provided to direct the users to generate various data reports like general report, year wise report, period wise report, period wise report of total shrimp waste etc. An administrative forms also designed for the admin to ease the process of data entry, editing and updating the data points at any point of time.

### Execution of Database Reports

The user interface helps to generate various types of reports to various queries. A Report in a database is the object for formatting, calculating, printing and summarizing selected data from the database. Reports can be generated from any table or query. Here,

reports are designed to retrieve customized database queries such as general report, year-wise report, period-wise report, period – HL-wise report, period – HLSO report, period – PD & PUD report and period – total shrimp waste report.

Sample period-wise report generated for the period from 2001 to 2010 is given in Figure 3. Buttons are provided to export the contents of report to external document formats in the forms such as PDF/ Microsoft Excel/ Microsoft Word, enabling the user to save reports to their local device. The database is available with the agricultural knowledge management unit (AKMU) of ICAR-CIFT, Cochin.

### User Panel



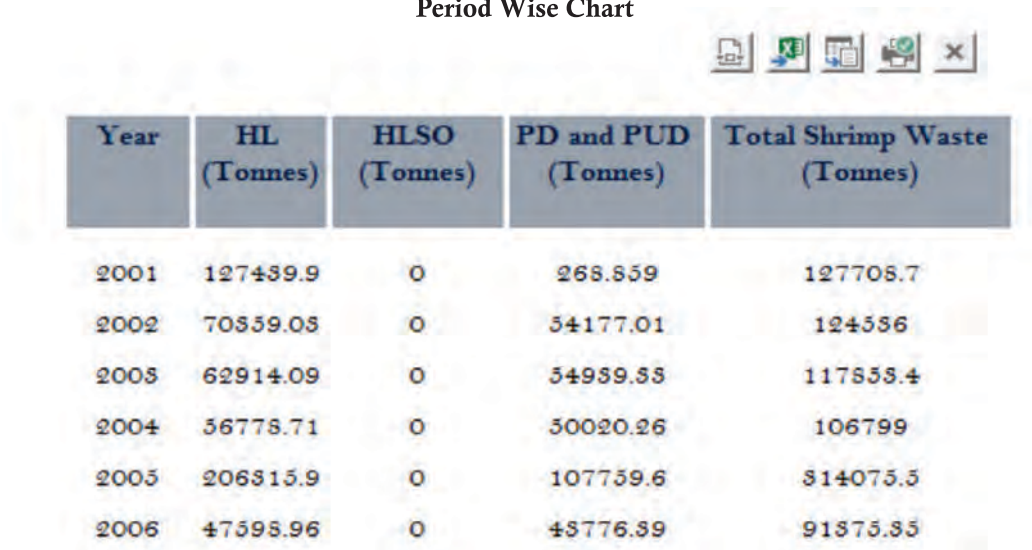
### Database on Shrimp Processing Waste from Organized Processing Sector in India

General Chart
Year Wise Chart
Period Wise Chart
Period - HL Chart
Period - HLSO Chart
Period - PD and PUD Chart
Period - Total Shrimp Waste Chart
Close

*Fig. 2 User panel Form of the database*



Period Wise Chart



Year	HL (Tonnes)	HLSO (Tonnes)	PD and PUD (Tonnes)	Total Shrimp Waste (Tonnes)
2001	127439.9	0	268.359	127708.7
2002	70359.03	0	34177.01	124536
2003	62914.09	0	34939.33	117853.4
2004	36778.71	0	30020.26	106799
2005	206315.9	0	107739.6	314075.5
2006	47593.96	0	43776.39	91375.35

*Fig. 3 Sample of period wise report***Reference**

[https://www.tutorialspoint.com/ms\\_access/ms\\_access\\_tutorial.pdf](https://www.tutorialspoint.com/ms_access/ms_access_tutorial.pdf)<https://www.javatpoint.com/microsoft-access>

# Staphylococcal cassette chromosome *mec* (SCC*mec*) typing of MRSA from retail market fish samples

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In India, aquaculture practices are fundamentally promoted by the government owing to its economic contribution and nutritive importance (Gogoi et al., 2015). Assam, with no exception, is one of the Indian states actively involved in aquaculture and contributing 2% of gross state domestic product (GSDP) (Sivaraman et al., 2021). Besides, the aquaculture is also sustained by the congenial climatic seasons and worthy ichthyofaunal diversity. Despite the several advantages, Assam has to depend on other Indian states to meet the fish demand owing to unsustainable utilization of aquatic resources. Additionally, the unsystematic farming practices followed by the farmers also resulted in the escalation of several bacterial diseases in aquaculture which in turn intensified the use of antibiotics. Recently, a study recorded the high prevalence of methicillin-resistant *S. aureus* (MRSA) animal source foods, pointing at the acute necessity of MRSA surveillance and the proper mitigation protocols in order to bring the MRSA widespread down (Bhowmik et al., 2021; Sannat et al., 2021).

*Staphylococcus aureus* is a versatile and successful human pathogen causing broad-range of diseases. It has already been acknowledged that the *S. aureus* infections, especially in people with underlying diseases, can result in the life-threatening bacteremia or sepsis (Rehm et al., 2008). For the past few decades, antibiotics have played crucial roles in controlling several deep-seated bacterial infections. In this context, the methicillin was truly magical to control the widespread infections caused by *S. aureus*. By contrast, the highly virulent *S. aureus* acquired methicillin-resistance and continued to be a global

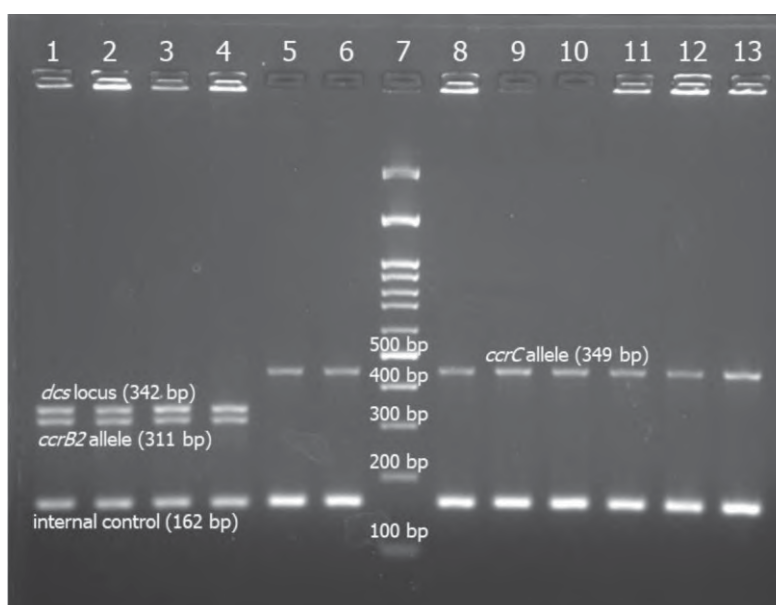
threat. Unfortunately, methicillin-resistance was observed among staphylococci including the aggressive *S. aureus* two years after the introduction of methicillin to the market. Owing to the high prevalence of methicillin-resistant *S. aureus* (MRSA) especially in India, the research interest on the epidemiology and the characteristics of MRSA has been intensified. The evolution of MRSA was facilitated by the acquisition of methicillin-resistance determinant namely *mecA* gene which is nested on staphylococcal cassette chromosome *mec* (SCC*mec*); a highly transmissible genetic island (Sekizuka et al., 2019). Notably, a structural homologue of *mecA* known as *mecC* sharing considerable sequence similarity has also been identified in MRSA sourced predominantly from livestock (García-Álvarez et al., 2011). Structurally, SCC*mec* has two major components such as *mec* gene complex and cassette chromosome recombinase (*ccr*) gene complex. Based on the difference in *ccr* and *mec* allotypes, MRSA has been classified to 11 SCC*mec* types (SCC*mec* I-SCC*mec* XI) (Kondo et al., 2007). Besides, the SCC*mec* I-III types are prevalent in MRSA sourced from healthcare settings (hospital-associated MRSA [HA-MRSA]) whereas SCC*mec* IV-V is carried by the community-associated MRSA (CA-MRSA) (Popovich et al., 2008). Now, SCC*mec* typing has become a gold standard for the epidemiological studies of MRSA. In view of this, the present study was carried out to understand the molecular epidemiology of fish-sourced MRSA by employing SCC*mec* typing.

Based on the ethnographic information on the aquatic resources, three study sites viz; Silagrant (site



1), Garchuk (site 2) and North Guwahati Township Committee (site 3) which are suspected to be the antimicrobial resistance (AMR) hotspots, were selected for sampling. 23 numbers of non-duplicate MRSA strains were isolated from 173 fish samples from the retail fish markets in site 1 (n=45), site 2 (n=54) and site 3 (n=74) in August 2019 and February 2020. The fish samples were collected in sterile polythene bag and transported to the lab in chilled condition. After macerating the fish samples, aseptically transferred to tryptic soy broth (TSB) containing 4% sodium chloride and 1% sodium pyruvate. Furthermore, the enriched culture is then serially diluted and inoculated to Mannitol salt agar (MSA) plates. The bacterial colonies with the typical morphology and colony characteristics of *S. aureus* (yellow colonies) were picked and screened for methicillin-resistance. Briefly, 10 µL of presumptive *S. aureus* bacterial suspensions were spot inoculated to Brain Heart Infusion (BHI) broth and incubated the plate at 35°C for overnight. Of 173 fish samples screened for methicillin-resistance, 95 samples carrying, *S. aureus* when subjected to methicillin susceptibility test, 23 isolates were recognized as MRSA. Moreover, MRSA were genotypically confirmed by the PCR amplification of 23S rRNA (*S. aureus* confirmation) and *mecA* gene (MRSA

confirmation). Then, 23 non-duplicate MRSA isolates were further subjected to molecular epidemiology by employing SCCmec typing (Sivaraman et al, 2021). Based on the PCR amplification of *ccr* and *mec* gene allotypes, MRSA is assigned with SCCmec types. In this study, PCR amplification of *ccrB2* allele (311 bp) and *dcs* locus (342 bp) was observed in 4 isolates (Fig. 1). Based on this, the 4 MRSA isolates are assigned with SCCmec type IV which is a molecular marker of CA-MRSA whereas, the remaining isolates contained only *ccrC* allele (449 bp) which is commonly found in type V elements but lacking *mec* gene complex alleles and thus type could not be assigned. Notably, ST22-EMRSA-15-IV and ST772-MRSA-V (Bengal Bay clone) are the major successful MRSA clones circulating in India. Among the several SCCmec types reported till now, type IV elements are structurally very small and thus contributing to the rapid dissemination of methicillin-resistance among staphylococci. The circulation of MRSA in different sectors is attributed to several known and unknown reasons. Majorly, personal hygiene of fish workers is very crucial to prevent post-harvest contamination of food fishes. Similarly, the imprudent use of antibiotics either as prophylaxis or growth promoters in aquaculture can also significantly contribute to the emergence of AMR, attested by several studies. In this



**Fig. 1** The PCR amplification of SCCmec elements in the MRSA. Lane 1-4 represents the MRSA carrying SCCmec type IV elements; Lane 5-6 & 9-13 represents the MRSA carrying *ccrC* allele; Lane 7 represents 1 kb gene ruler

context, this study identified the importance of personal hygiene to be followed by the fish workers since the samples for the present study was mostly collected from retail markets. Considering the one health, the study also highlighted the necessity of continuous MRSA surveillance especially in fishery sectors.

### Funding

This study was supported by Department of Biotechnology (DBT), Government of India (BT/IN/indo-UK/AMR/06/BRS/2018-19) and Economic and Social Research Council, UK.

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# Simplified scheme for isolation of broad host range bacteriophages and *in vitro* assay for optimizing multiplicity of infection against AMR *E. coli*

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Worldwide consumers relish fish as food, owing to its nutritional and health benefits. Fish and fishery products remain an excellent and affordable source of animal protein and are being recommended for variety of deficiency associated diseases. The fish production of India in 2019-20 was 141.6 lakh tonnes of which inland fisheries contributed 73.7% and marine capture fisheries contributed 26.3% indicating that the aquaculture was predominantly contributing to the fish food basket and is expected to remain the same in the immediate future. Together the contribution of carps, tilapia and farmed shrimp is estimated to exceed 50% of the aquaculture production of farmed animal (FAO, 2020). Bacteria impact the health of the aquatic animals during aquaculture and potentially affect public health due to

improper post-harvest handling practices.

Bacteriophages are viruses that have the ability to lyse bacteria and in the wake of antimicrobial resistance they are being repurposed as natural antimicrobials for control of aquatic animal bacterial pathogens and human pathogens. Even though bacteriophages that have the ability to lyse a broad range of bacterial isolates are instrumental for successful phage therapy, the isolation and characterization methods are very cumbersome. In the present report, a simplified scheme is proposed (Fig. 1) with protocols for the isolation of broad host range phages and estimation of multiplicity of infection for selection of broad host range bacteriophages for phage therapy.

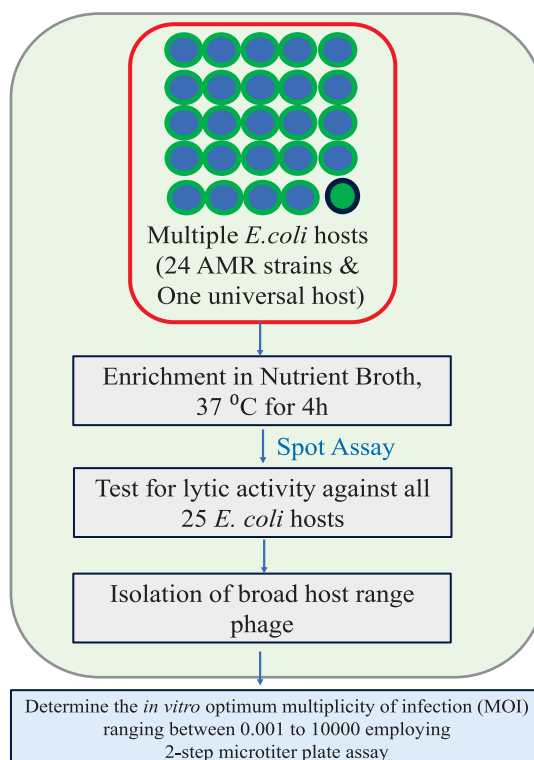


Fig. 1 Simplified scheme for isolation of broad host range phages and quick optimization of multiplicity of infections

The first part of the scheme is to isolate broad host range bacteriophages using a recently developed multi-host enrichment procedure that simultaneously employs 25 isolates of *E. coli* as bacterial hosts (Vaiyapuri *et al.*, 2021) instead of the conventional single host enrichment method that uses only one *E. coli* isolate as host. The multiple-host enrichment method involves the following steps.

- Collect the sample for phage isolation, preferably from the environment where the target bacterium dwells.
- Grow each of the 25 isolates of bacteria separately in 2mL of nutrient broth (NB) and incubate at  $35 \pm 2^\circ\text{C}$  for 16h.
- Add 500 $\mu\text{L}$  of the culture from each NB tube ( $n=25$ ) and 47.5mL of the sample to a flask containing 15 mL of 5x NB, mix well and incubate at  $35 \pm 2^\circ\text{C}$  for 4h.
- Filter (0.45  $\mu\text{m}$ ) the enriched culture and test the filtrate for lytic activity, separately against all the 25 bacterial isolates by spotting (Fig. 2) method (Kutter, 2009).
- Separate individual phages from the filtrate by the single agar method (Rao and Surendran,

2003), re-enrich in the respective host culture, filter and store ( $-20^\circ\text{C}$ ) the phage rich filtrate for further studies.

The second part of scheme is the 2-step assay employing 96 well microtitre plate for determining the optimum multiplicity of infection (MOI) (Benala *et al.*, 2021), wherein nine different *in vitro* MOIs ranging between 0.0001 and 10000 were tested in the first step and the optimum MOI (lowest quantity of phage) for inhibiting the target bacteria was obtained in the second step. This optimum MOI of the phage may then be tested as the starting dose for therapeutic applications. The MOI selection is performed in a 96 well microtiter plate placing 300  $\mu\text{L}$  medium in each well containing 240  $\mu\text{L}$  of nutrient broth, 30  $\mu\text{L}$  of bacteria (different concentrations of bacteria prepared from 0.3 OD<sub>600</sub> culture) and bacteriophage (different concentrations prepared from stock stored at  $-20^\circ\text{C}$ ) in three replicate wells. Media control, phage control and bacteria control are included in three replicate wells. The absorbance (OD at 600 nm) was measured in each well at 30 minutes interval for 4 h. The lowest MOI that prevented growth in the first step is selected for optimization in the second step. MOI optimization is performed using the lowest MOI of first step and intervening MOI between the lowest and next highest

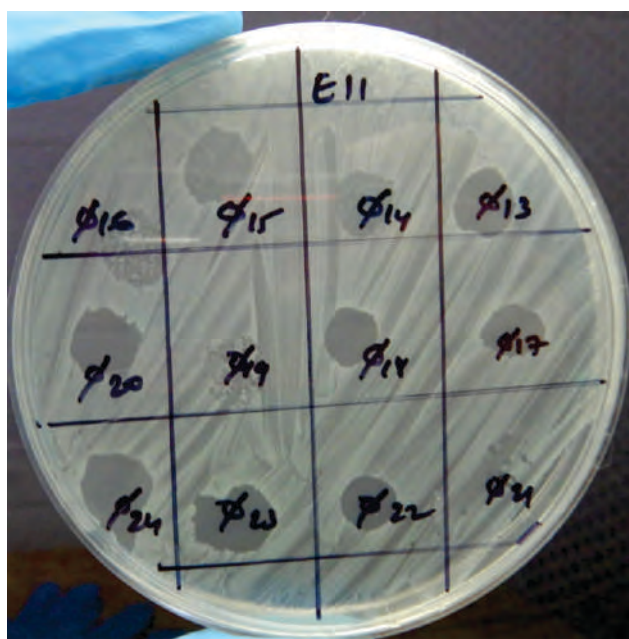


Fig. 2 Detection of lytic activity of bacteriophages by spotting method



MOI of first step. The result of the 2-step microtiter assay helps to arrive at the lowest quantity of phage required for preventing log phase growth of the target bacteria.

This updated simplified scheme helps in augmenting phage repositories with broad host range lytic phages with optimized *in vitro* MOIs for phage therapy in human health care, agriculture and animal agriculture.

#### Acknowledgement:

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# Opportunities of seaweed value addition and quality control requirements in India

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## Introduction

Seaweeds has long documented history of uses as food in different parts of the world. Now, backed by recent research, seaweeds are perceived as superfood, rich in protein, micronutrients, vitamins, and health promoting phytochemicals. Globally, seaweeds are traded as ready to eat food and functional food products with a premium price tag. Besides, the global seaweed trade is also driven by the prominent seaweed hydrocolloids, namely Agar, Alginate, and Carrageenan. Markets for seaweed-based cosmetics, animal feed ingredients, fertilizers, soil conditioners are relatively small but rapidly growing. Dried seaweeds as raw materials in various industries are also traded but fetches comparatively lower price. Hence, besides ramping up seaweed production, judicious and focused investment for establishing post-harvest infrastructure and value chain is essential.

The Indian coastline of 8100 km and EEZ of 2.17 million km<sup>2</sup> support luxuriant growth of diverse seaweed resources belonging to 700 species. Of these, nearly 60 species are economically important. Approximately 20,000 t (wet weight) of these resources are harvested annually, while the actual harvest potential is close to 870,000 t. The major species harvested are *Gracilaria edulis*, *Gelidiella acerosa*, and *Kappaphycus alvarezii* among red algae; *Sargassum wightii*, *Turbinaria conoides*, and *Cystoseira* spp. among brown algae and *Ulva lactuca*, *Enteromorpha* sp., *Caulerpa* spp. among green algae. The state-wise distribution of number of seaweed species in the country are, Gujarat 202; Maharashtra 152; Goa 75; Karnataka 39; Kerala 20; Lakshadweep 89; Tamil Nadu 302; Andhra Pradesh 78; Orissa 1; West Bengal 6 and Andaman & Nicobar Islands 34. These rich resources of seaweed in India could potentially

serve and benefit the functional food, pharmaceuticals, textiles, fertilizers, and animal feed industries.

## Market potential

The global seaweed industry is valued at approximately US\$ 5.5 – 6 billion, out of which products intended as food contribute about US\$ 5 billion (FAO, 2018). China, the European Union, Japan, and the USA are the major importers of seaweed products. In 2016, top 35 seaweed importing countries traded in seaweed products worth USD 2 billion. Carrageenan and edible seaweed products were the major imported items with a share of 37% and 30% respectively. The market analysis indicates that besides seaweed hydrocolloids, market for edible seaweed products is equally lucrative. The market for high value seaweed nutraceuticals such as fucoidan and fucoxanthin is rapidly growing. Fucoidan is a bioactive sulphated polysaccharide found in brown seaweed. Many research reports noted the anti-inflammatory, anti-viral, and anti-cancer activities of fucoidan. Fucoxanthin is a carotenoid found in brown seaweed. The carotenoid has proven anti-diabetic and anti-obesogenic properties. A recent market research suggests that the value of global “Fucoidan Market” in 2020 is 30 million USD and growing at a compound annual growth rate (CAGR) of 3.8%. While, the global “Fucoxanthin market” is valued at 95 million USD in 2020, growing at a CAGR of 2.6%.

## Quality control of seaweed raw material for food and feed purpose

The seaweed raw materials used for food and feed purposes need to be tested for Iodine and Mercury. In 2006, the European Union (EU) for Scientific Committee on Food (SCF) established an upper limit of 600 µg/day for iodine intake for adults and 200



µg/day for children of 1-3 years of age. For mercury in algae and prokaryotic organisms, a maximum residue level of 0.01 mg/kg is established according to Regulation (EC) No 396/2005. For arsenic, lead, cadmium, and mercury, the maximum levels in the feed are established under EU Directive 2002/32/EC of the European Parliament and the Council. Metal content in seaweed is important from animal health care and food safety point of view as many seaweed species are used as animal feed or feed ingredient. As per this EU directive 2002/32/EC Aldrin, Dieldrin, Toxaphene, Chlordane, DDT, Endosulfan, Endrin, Heptachlor, Hexachlorobenzene, and Hexachlorocyclohexane needs to be tested. The regulatory limits for feed material/ ingredients are presented in Table 1.

For polycyclic aromatic hydrocarbons and polychlorinated biphenyls such regulatory limits are not available. However, the presence of these organic pollutants is a possibility in seaweeds and should be monitored. In this case, a default regulatory limit of 0.01 ppm can be considered (Regulation (EC) No 396/2005).

In India, as of now, there is no regulatory limit for heavy metals and persistent organic pollutants in seaweed for food supplement and feed purpose. The Food Safety and Standards (Contaminants, Toxins, and Residues) Regulations, 2011 mentions a regulatory limit for Mercury in non-specified food as 1 mg/kg and Methyl mercury in all food stuff at 0.25 mg/kg. The same should be applied to seaweed-based food and supplements. More importantly, the Gazette of India Notification No. 465 on Food Safety and Standards (Health Supplements, Nutraceuticals, Food for Special Dietary Use, Food for Special Medical Purpose, Functional Food and Novel Food) Regulations, 2016 mentions only “Kelp” as an approved nutraceutical or supplement ingredient in India. No other edible Indian seaweeds are listed. This Gazette notification should be amended to include Indian edible seaweed species to legally market seaweed-based supplements and nutraceuticals in India. The probable enforcing agency for seaweed-based food and supplements may be the FSSAI. Whereas for seaweed-based feed the Ministry of Agriculture and Farmers Welfare may be the enforcing agency.

**Table 1.** Regulatory limits for feed material/ ingredients (Source: EU Directive 2002/32/EC)

Parameter	Tolerance limit (mg/kg at moisture of 12%)
Arsenic	2.00
Lead	10.00
Mercury	0.10
Cadmium	1.00
Aldrin and Dieldrin (Single or combined)	0.01
Toxaphene	0.10
Chlordane	0.02
DDT	0.05
Endosulfan	0.10
Endrin	0.01
Heptachlor	0.01
Hexachlorobenzene	0.01
α-HCH	0.02
β-HCH	0.01
γ-HCH	0.20

### High-value food additives from seaweeds

Regulatory limits for heavy metals have been mentioned in European Commission Regulation (EU) No 231/2012 of 9 March 2012 for high-value food additives from seaweed. Formaldehyde (50 mg/kg), Arsenic (3 mg/kg), Lead (2 mg/kg), Mercury (1 mg/kg), and Cadmium (1 mg/kg) should be monitored. The values in parenthesis indicate maximum permissible limits. *E. Coli* should be absent in 5 g, and *Salmonella sp.* should be absent in 10 g. In India, the Food Safety and Standards (Food Products Standards and Food Additives) Regulation, 2011 mentions regulatory limits for Agar, Alginates, and Carrageenan. For Agar and Alginate, the Lead and Arsenic content should be no more than 5 and 3 mg/Kg respectively. For Carrageenan, regulatory limits of Cadmium (1.5 mg/Kg), Mercury (1 mg/Kg), Arsenic (3 mg/Kg), and Lead (5 mg/Kg) have been specified. *E. Coli* and *Salmonella sp.* Should be absent. The probable enforcing agency may be the FSSAI for high-value food additives from seaweed.

### Quality control of seaweed fertilizer and plant growth regulators

Levels of heavy metals and pesticides in seaweed-based bio-stimulants should be monitored as per the Gazette of India Notification No.812 (CG-DL-E-24022021-225410) of the Ministry of Agriculture and farmers welfare. The gazette notification specifies maximum permissible levels of Cadmium, Chromium VI, Copper, Zinc, Lead, and Arsenic as 5 mg/kg, 50 mg/kg, 300 mg/kg, 1000 mg/kg, 100

mg/kg, and 10 mg/kg respectively. For pesticides and other organic pollutants, the permissible limit is 0.01 ppm. Seaweed-based plant growth regulators contain micronutrients, amino acids, and plant growth regulators (auxins, cytokinins, polyamines, gibberellins, abscisic acid, and brassinosteroids), phloroglucinol and eckol, etc. Label claims for these constituents should be there and the products should be analyzed to verify the label claim. The probable enforcing agency may be under the Fertilizer Control Order. State Governments are responsible for enforcing the fertilizer control order. Central Fertilizer Quality Control & Training Institute and its three Regional Laboratories, State fertilizer testing laboratories should be responsible. ICAR institutes may extend support for quality analysis of seaweed bio-stimulants.

### Conclusion

ICAR-CIFT has developed several technologies for value added products and nutraceuticals from seaweed. Five of the technologies have been transferred to industries and commercial production has started for four of them. ICAR-CIFT is the national reference laboratory of FSSAI for fish and fisheries products and will have important role to play for quality control of seaweed and seaweed products. The institute has pilot plant facility for process demonstration and have transferred the technology for solar dryers for hygienic drying of fish and fish products. However, a conducive policy environment needs to be formulated for achieving the true potential of seaweed value addition and processing in India.



# Design of a solar powered automatic fish feed dispenser for aquaculture ponds

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In recent years, aquaculture has grown immensely due to the huge demand for fish as protein source for human. Aquaculture activity majorly contributes to fulfil the total fish requirement of nation/globe. Feeding is one of the most important activities of fish production in aquaculture. Feed is an important input for aquaculture and in smaller farms feed dispensing done manually which is a very labour intensive and challenging activity (Yeoh *et al.*, 2010). Excess feeding leads to wastage of feed and causes water pollution, thereby diseases in fish. Manual fish feeding is very time consuming, especially for large ponds. Automatic feed dispensing systems are necessary to overcome these challenges and to enhance the fish production efficiency.

Automatic fish feeders are electronic devices which feeds the fish with predetermined amount of food at certain time. These devices are helpful to reduce manual work and save labour time in fish feeding practices. Low cost automatic accurate devices are the best option to replace the manual feeding method (Uddin *et al.*, 2016). Hence, there is a need to develop an automatic feeder along with

floatation unit to efficiently dispense feed at each corner of the pond to minimize the loss. It was envisaged that to design and develop a power-free, inexpensive, automatic fish feed dispenser for aquaculture ponds.

Solar powered automatic fish feed dispensing system (Fig.1) is an electronic device that is designed to discharge fish feed at a specific time each day in all directions of the pond. It helps in increasing the efficiency and productivity in fish farming field in long term. It helps aquarist to feed fish in their absence and to maintain a regular feeding schedule. Feed dispensing system uses a single board micro controller, an open-source micro controller board based on ATmega328P micro controller which allows the user to control the feeding time using 4\*4 keypad and thus the amount of feed to be dispensed at each time. The amount of feed discharge depends on the duration of feeding time which can be set in programme. It also helps to prevent overeating by releasing the right quantity of food, at scheduled times and assures well fed, healthy fishes.

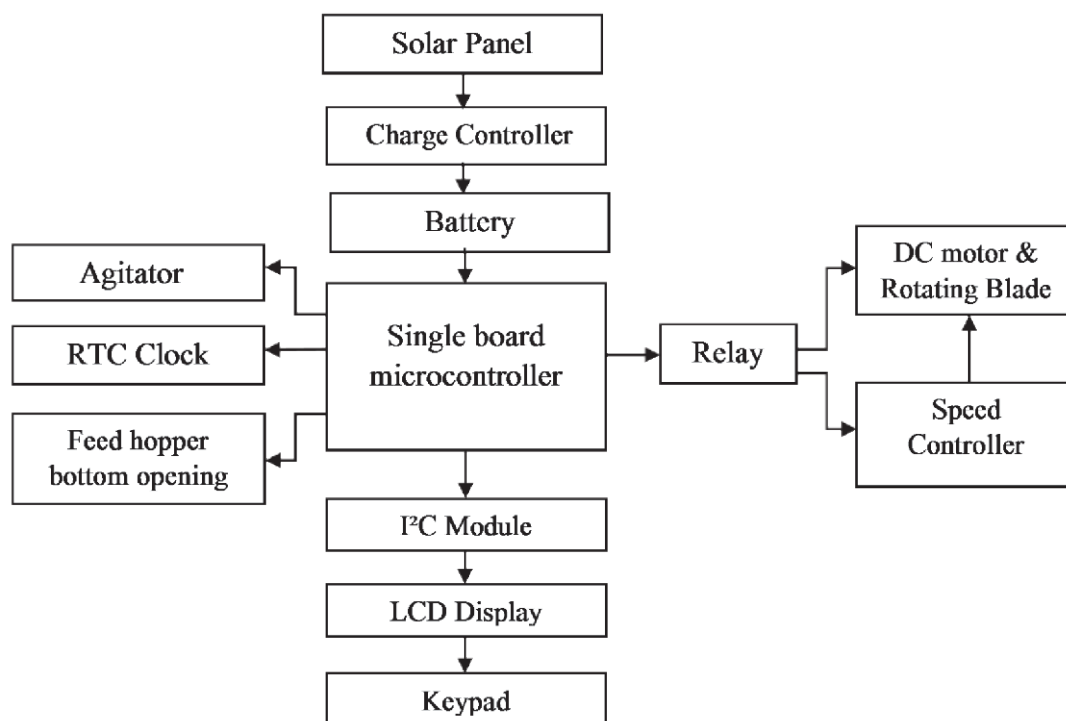


**Fig. 1.** Solar powered automatic fish feed dispensing system

The solar powered automatic fish feed dispensing system consists of a feed hopper made of aluminium sheet which can hold 6 kg of feed. An agitator is placed inside the feed hopper to avoid clogging of feed in discharge end of feed hopper and it is run by a servomotor of 6W. Hopper bottom is closed using acrylic sheet and the opening and closing of hopper bottom is controlled by another servomotor of 6W. Acrylic sheet is having various sizes of opening which helps in the control of quantity of feed discharge. The Fish feed dispensed from the hopper will be dropped over a rotating blade connected with the shaft of DC motor. Rotating blade dispenses the feed equally in all directions and the rotation speed of blade can be controlled by regulating the voltage of DC motor. Variation in rotation speed influences the amount of feed dispensed and feed spreading area.

Feed dispenser has a solar panel (30 W) that converts sunlight into electricity and then stored in a

12 volts 7 Ah lead acid battery. The 12 V DC serves as the power source to run DC motor and two servomotors. The 10 A charge controller acts as a filter of excessive charging of the battery which prevents overloading and prolonging the battery's lifespan. The feeding durations and feed-time intervals are coded as separate programs called sketches which are then compiled into the single board micro controller using an integrated development environment (IDE). A highly sophisticated program has been written on the board for carrying out the overall functionality. A 16\*2 LCD display, real time clock module and keypad are provided for that allows the user to set the time on a 24 h time format as and when feeding is required. The solar powered automatic fish feeder device also has HDPE float system that helps in even distribution of feed among the pond with less wastage of feeds.



*Fig. 2 Block diagram of solar powered automatic fish feed dispenser*

A solar powered automatic fish feed dispenser was developed using a single board microcontroller which dispense the predetermined amount of feed at particular time every day. This system is a combination of mechanical and electrical devices and consists of solar panel, feed hopper, feed dispensing rotating plate, DC motor, battery, charge controller, keypad, Real Time Clock module, LCD display and high-density polyethylene (HDPE) aqua float.

Experimental results revealed that the fish feed dispenser can able to distribute 250 g of 1.2, 2.5 and 5 mm size pellets in 9, 12 and 33 sec to the longest distance of 4.6, 5 and 6.8 m at the blade rotation speed of 3000 rpm, respectively. The overall feeding efficiency of the feed dispenser was observed as 92-97.4 %. This equipment was found to be easy to handle and operate, inexpensive and profitable than other manual feeding system.

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# Estimation of energy efficiency of CIFT solar hybrid dryers

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**I**CAR-Central Institute of Fisheries Technology, Kochi has developed energy-efficient, low-cost and eco-friendly solar hybrid dryers for hygienic and uniform drying of fish and fishery products (Fasludeen et al., 2018). A solar hybrid dryer can generate higher air temperatures followed by lower relative humidity, which favors improved drying rates and lower moisture content of the final dried products. Global solar energy generation was estimated to reach a rate of 8.9% annually between 2012 and 2040, creating a way for faster energy generation for various applications (Kumar and Singh, 2020). Thus, solar hybrid drying technology can be an ultimate solution to achieve energy-efficient drying as drying of agricultural products consumes around 3.62% of the world's total energy consumption (Mohana et al., 2020). This article discusses the details of eco-friendly, cost-effective, and hygienic solar driers developed by CIFT.

The solar electrical hybrid dryer of 20 kg capacity comprised of solar flat plate collectors with an area of 10 m<sup>2</sup> for harnessing solar energy to heat the air (Fig.1). The hot air was circulated into the drying chamber for drying the product (Alfiya et al., 2018). In the absence of solar radiation during cloudy/rainy days to heat the air for circulation, alternative electrical backup system will be automatically activated. Drying was carried out under controlled temperature and humidity conditions.

Solar electrical cabinet dryer with 40 kg capacity

consisted of thirty-six trays, nine each in four chambers inside the dryer (Fig.2). A through-flow drying pattern was accomplished inside the drying chamber by the perforated trays placed at a distance of 10 cm vertically. Solar flat plate collectors harnessed solar energy and transferred it to the air flowing through the collector which was then transmitted to the drying chamber (Murali et al., 2019). During rainy or cloudy days, when desired temperatures were not attained in the drying chamber, electrical backup will be automatically activated.

Solar LPG hybrid dryer is a solar drying system for the hygienic production of dry fish by using environment-friendly, abundantly, freely available and renewable solar energy. Continuous drying of fish is possible in this system with the help of LPG backup, where the fish can be dried in unfavorable weather conditions without spoilage and maintaining its nutritional value. The hot water from the calorifier tank was collected through the solar collectors using a pump. Axial flow fans were provided in the drying chamber for hot air circulation across stainless steel trays loaded with fish for drying. The circulating air was heated by hot water passing through the heat exchangers (Murali et al., 2020; Murali et al., 2021). LPG backup heating system supplemented the heat requirement for drying during rainy or cloudy days to heat the water for circulation (Fig. 3). The complete process parameters of fish drying could be controlled by a PLC system.



Fig. 1 Solar-electrical dryer - 20 kg



Fig. 2 Solar-electrical cabinet dryer - 40 kg



Fig. 3 Solar-LPG-electrical dryer - 50 kg

Table 1. Energy consumption charges for CIFT developed solar hybrid dryers

Sl. No.	Type and capacity of the dryer	Cost of the dryer (in Rs.)	Operational cost of dryer Rs./kg fish	Drying time (h)*	Manpower requirement for handling	Electricity/ LPG charges for one year (in Rs.)**	LPG charges for 1 year (in Rs.)**
1	Solar-electrical hybrid dryer - 20 kg	1,50,000 + 18% GST	2.9	6-10	1	14,361	Nil
2	Solar-electrical hybrid dryer - 40 kg	2,80,000 + 18% GST	2.15	6-10	2	20,688.3	Nil
3	Solar -LPG-electrical hybrid dryer - 60 kg	4,20,000 + 18% GST	3.65	6-10	2	41,823.7	10,800
4	Solar-LPG - electrical hybrid dryer - 250 kg	13,75,000 + 18% GST	2.11	6-10	4	83,647.4	43,200

\* vary depending on the type and thickness of fish

\*\* Considering 8 months of operation @ 1 batch per day (1 batch approximately equivalent to 8 hours) and unit cost of electricity as Rs. 8/- Assuming 30% of energy supply through heating coils/LPG and 70% by solar collectors during daytime

The energy consumption chart of CIFT developed solar hybrid dryers is depicted in Table.1, and a comparison chart among solar hybrid and electrical/LPG dryers is given in Table.2. It is evident

from the table that the solar hybrid dryers are energy efficient with a reduction in energy expenses to the tune of 40-60%.

**Table 2.** Comparison of energy consumption of solar hybrid, electrical and LPG dryers

Type of the dryer and capacity	Total energy consumption (KWh) for 1 year (240 days)	Approximate electricity and LPG charge for 1 year (in INR)	Percentage reduction in energy	Percentage reduction in cost
Solar Electrical Hybrid - 20 kg	1795.20	14, 361	59.95	59.95
Electrical - 20 kg	4483.2	35,865.60		
Solar Electrical Hybrid - 40 kg	2586.04	20,688.38	60.92	60.92
Electrical - 40 kg	6618.64	52,949.12		
Solar LPG Hybrid - 60 kg	5227.96	52,623.70	42.34	51.5
LPG - 60 kg	9067.96	1,08,543.74		
Solar LPG Hybrid – 250 kg	10,455.93	1,26,847.48	42.34	56.12
LPG dryer - 250	18,135.93	2,89,087.44		

It was found that all CIFT developed solar-based hybrid drying systems were energy-efficient and promote conserving electricity or fossil fuels. The

solar dryers work on minimal operating expenses and provide better quality hygienic products.

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