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Aims and Scope

AQUATIC RESEARCH

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Editor in Chief:

Prof. Dr. Nuray ERKAN

Address: Istanbul University, Faculty of Aquatic Sciences, Department of Food Safety, Kalenderhane Mah. 16 Mart Şehitleri Cad. No: 2, 34134 Fatih/Istanbul, Türkiye

E-mail: nurerkan@istanbul.edu.tr

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Aquat Res 6(4), 233-244 (2023) • https://doi.org/10.3153/AR23022 *Research Article*

Anatomy and histological evaluation of the reproductive system of marine calanoid copepod *Centropages furcatus* from a mass culture perspective

M. Asrar SHERIFF[1], Vijayaraj RADHA[2], Kareem ALTAFF[1, 2]

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[1] The New College, Department of Zoology, Chennai – 600014, India.

[2] AMET University, Department of Marine Biotechnology, Aquaculture Research Laboratory, Chennai – 603112, India.

ORCID IDs of the author(s):
M.A.S. 0000-0002-3562-2534
V.R. 0000-0002-4170-1685
K.A. 0000-0001-9178-2519

ABSTRACT

Marine finfish culture is a priority area for future human food security. Although many marine finfish species have been domesticated and successful breeding and spawning in captive conditions are achieved, the larval rearing from hatchling to fingerling stage to the desired level is yet to be achieved. One of the essential impediment factors in this process is the availability of suitable live feed for the finfish larvae. The traditional live feeds (*Artemia* nauplii and rotifers) must be more adequate in size spectrum and nutritive value to many marine finfish larvae. In nature, copepod nauplii, copepodite stages, and adults constitute the preferred food of marine finfish larvae. Copepod size spectrum, nutritive value and swimming movements make them ideal live prey items for fish larvae. Nevertheless, the high-density culture of copepods is challenging due to their sexual reproduction and high species-specific variability. Further, adequate knowledge of the candidate species' food and feeding habits, reproductive biology, and life cycle strategies should exist. With this objective present study describes the female and male reproductive system, oogenesis and spermatogenesis, egg production, and reproductive potential of a candidate calanoid copepod species, *Centropages furcatus.* This basic information will help develop mass culture protocol for this species.

Keywords: Copepods, Reproductive system, Anatomy, Histology

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Correspondence:
Kareem ALTAFF
E-mail: kaltaff@gmail.com

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Introduction

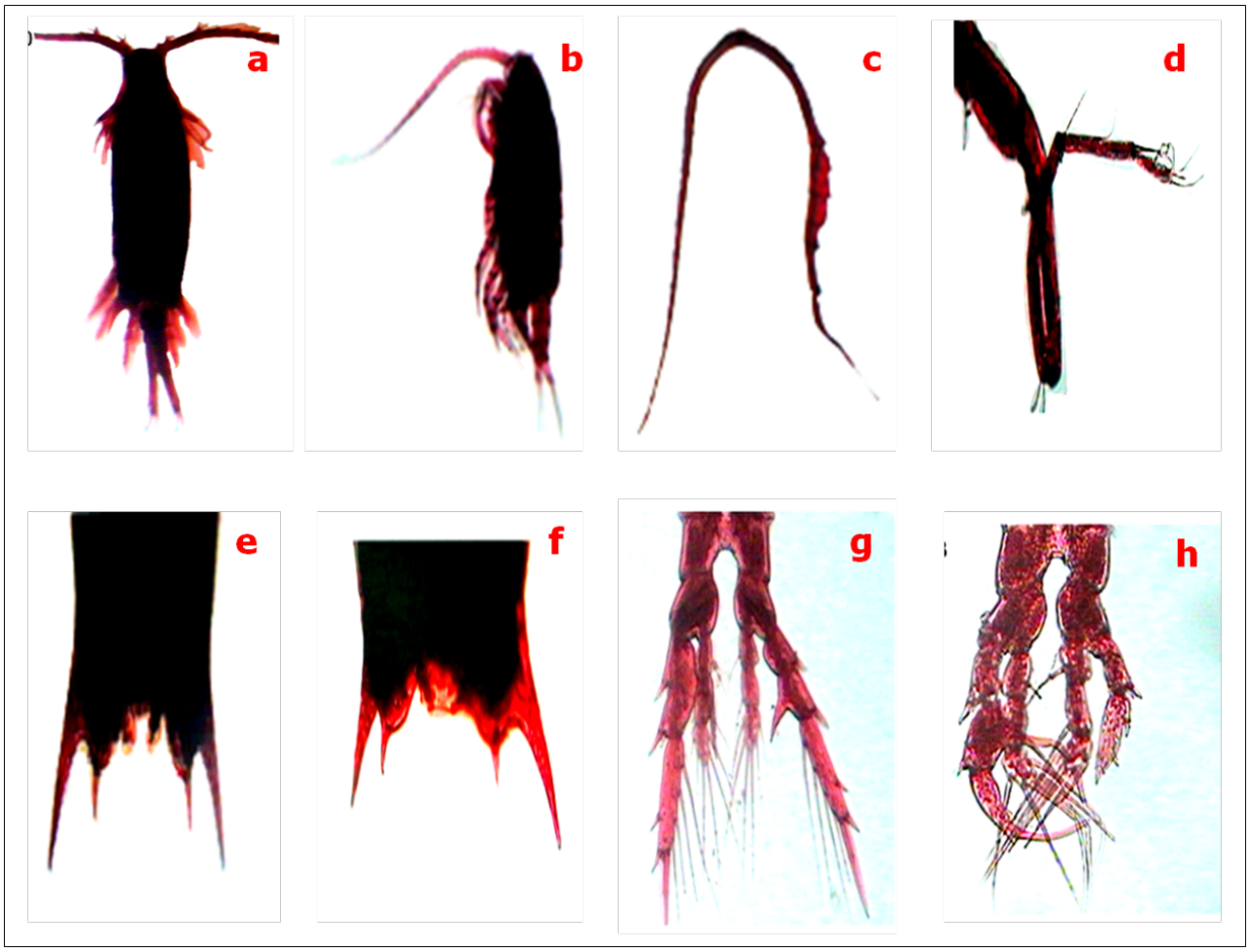
Coastal aquaculture and mariculture are essential in many developing countries' livelihoods, employment, and local economy. Mariculture is practised in areas adjacent to the sea, such as land ponds along the coast and closed lagoons. Advancements in breeding technology, disease management, feeds, and nutrition are vital areas in this field that can improvise and increase efficiency (Araujo et al., 2022). According to FAO (2022) 2020, farmed finfish reached 57.5 million tonnes in 2020, including 49.1 million tonnes from inland aquaculture and 8.3 million tonnes from mariculture in the sea and coastal aquaculture on the shore. Despite the high diversity of marine finfish cultivable species, mariculture contributes far less than inland aquaculture. Although broodstock of many marine finfish is developed and successfully made to spawn in captivity, the desired level of larval rearing for their farming is yet to be achieved (Kailasam et al., 2020). One constraint in the hatchery rearing of finfish larvae is the availability of suitable live feed for different larval stages. The live feed's size spectrum and nutritive value determine the larvae's growth, metamorphosis, and survival. The traditional live feed currently used in finfish larval rearing is rotifers and *Artemia* nauplii, which need improvement to support larval growth and survival. The life cycle of calanoid copepods from tropical marine waters shows six naupliar and six copepodite stages, the sixth being the adult. The body length of the post-embryonic stages from the first nauplius to adult ranges between 95µm to 1680 µm. Further, they contain higher polyunsaturated fatty acids, particularly docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), compared to rotifers and *Artemia nauplii*. Copepods constitute the primary food source for finfish larvae in nature, and many reports indicate better performance of finfish larval production in hatcheries when copepods are used as live feed (Rajkumar, 2006; Conceição et al., 2010; Ajiboye et al., 2011; Ma et al., 2013; Rønnestad et al., 2013; Kline and Laidley, 2015; Barroso et al., 2015; Burgess et al., 2020; Vijayaraj et al., 2022). Although many published reports have been on laboratory-scale mass production of copepods, achieving the commercial scale required for commercial finfish larval rearing is still to be accomplished (Imelda et al., 2015; Santhosh et al., 2018). Significant issues in developing copepod high-density culture include species-specific dietary requirements and reproductive patterns (Altaff, 2020). The reproductive potential and survival of copepods may have an unfavourable impact if a suitable diet and physicochemical parameters of the culture medium are not provided. In developing copepod culture, essential aspects of cultivable species required include sex ratio, egg production, egg hatching success, development of nauplii and copepodite stages, and population growth (Altaff and Vijayaraj, 2021). Copepods were the most appropriate live prey for sustainable culture practices of many marine finfish with small mouth sizes and nutritional requirements. The present study reports the reproductive biology of a potential calanoid copepod, *Centropages furcatus*.

Material and Methods

Zooplankton samples were collected offshore of the Kovalam station in Chennai (13.0827° N,80.2707° E) and preserved in 5% buffered formalin. The calanoid copepod *C. furcatus* was separated from the zooplankton sample and identified at the species level (Kasturirangan, 1963; Conway *et al.,* 2003; Lacuna et al., 2013). Live male and female *C. furcatus* were separated and transferred into 2L beakers containing filtered seawater. They were maintained with a mixed algal diet consisting of *Isochrysis* sp.*, Chaetoceros muelleri,* and *Chlorella marina*. To examine the female and male reproductive system of *C. furcatus in situ*, borax–carmine stained and acetic acid differentiated specimens were dissected in glycerol–ethanol mixture under a stereoscopic dissection microscope (Pantin, 1964). The female and male reproductive systems of *C. furcatus* were described using the terminology of Hopkins (1978) and Dussart and Defaye (1995). For histology, the specimens were fixed in aqueous Bouin's fluid, dehydrated in ethanol, cleared in xylene, and embedded in paraffin wax. Serial sections (cross sections and longitudinal sections) of 8 µm were cut and stained with haematoxylin and then counterstained with alcoholic eosin (Jeevaji *et al*., 1983). The different parts of the reproductive system were studied under a compound microscope and photomicrographed at magnifications of 100x and 400x. The live male and female *C. furcatus* collected from the wild were domesticated by raising them in 20L beakers for several generations with a microalgal diet to obtain desirable stock. To study their life span and fecundity, ten batches of males and females were maintained in 2L beakers, and survival and fecundity were followed for 38 days and recorded. Individual adult females and males were maintained in 500 mL beakers and constantly observed for mating and spawning. The number of eggs spawned was counted under a stereoscopic dissection microscope, and the mean of ten spawning was recorded. The body length of the six naupliar and prosome lengths of six copepodite stages of *C. furcatus* was measured under a stereoscopic dissection microscope (Magnus: MSZ-TR with Magcam D series) using ocular micrometre and a mean of ten measurements was recorded.

Results and Discussion

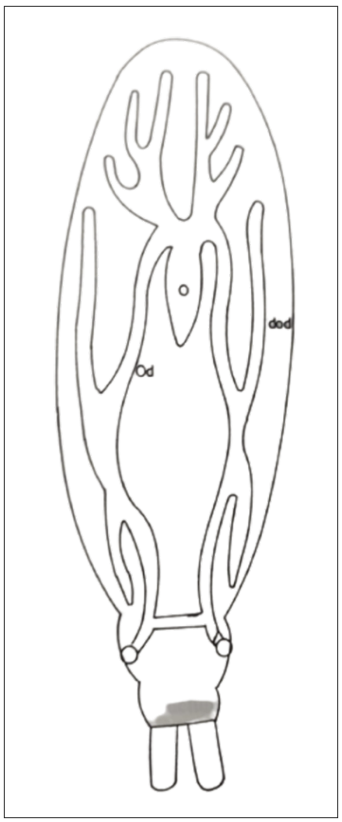
The transparent body of *C. furcatus* has an extended, slender anterior portion. Figures 1a and b show the male and female species' lengths, respectively, at 1.4mm and 1.6mm. This species' antennule, metasome, and P5 exhibit apparent sexual dimorphism in both sexes. According to Figures 1c and d, the male's right antennule is geniculate, with numerous expanded segments and articulation intended to grip the female during copulation. In contrast to the male's, the female's metasomal wings are symmetrical (Figures 1e and 1f). A rounded protrusion is present at the proximal area of the extension on the second exopodite segment of the right P5 of the male. In contrast to the male P5, which is asymmetrical and adapted to deliver spermatophore to the female vaginal pore, the female P5 is symmetrical (Figures 1g and h).



Figures 1. a. Adult female; b. Adult male; c. Antennules of male; d. Distal region of geniculate antennule; e. Metasomal spines of female; f Metasomal spines of male; g P5 of Adult female; h P5 of Adult male

Anatomy of the Female Reproductive System

The median ovary is 460 ±16 µm in length, and the pair of genital ducts make up the female reproductive system of *C. furcatus*. The oviduct takes a posterior route and travels near the ovary before diverting towards the lateral side of the prosome. The genital ducts emerge anterolaterally from the ovary and give rise to the anterior diverticula. They continue till the prosome's tip, at which point they expand into the genital segment and open to the outside. The oviduct produces Diverticulae, which occupy the posterior-lateral portion of the prosome. The genital segment has two seminal receptacles (Figure 2).



(*dod - diverticular of oviduct, o - ovary, od – oviduct*)

Figure 2. Female Reproductive system

Histology of the Female Reproductive System

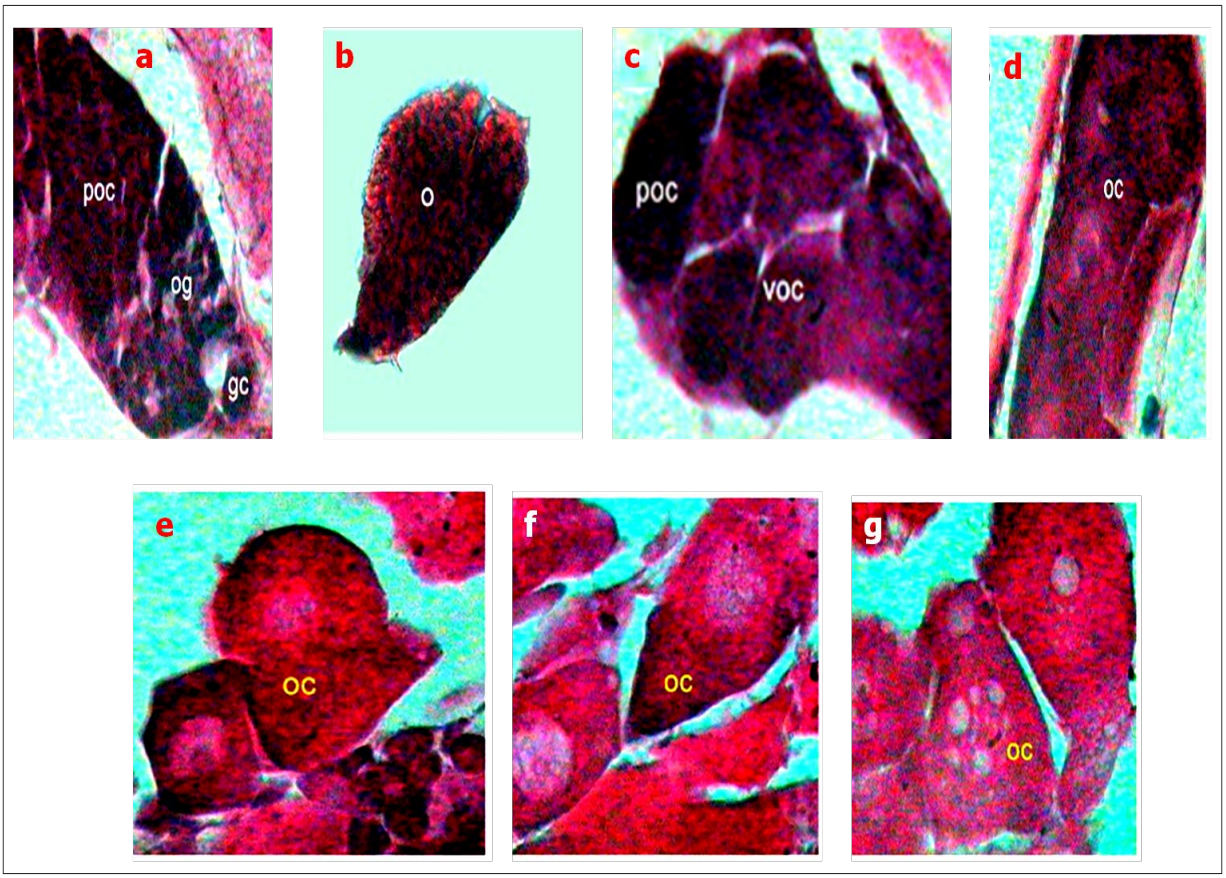
The ovary of the *C. furcatus* has a thin wall with epithelium of rectangular cells and a connective tissue layer. Different phases of oogenesis are grouped in ascending rows up to the front end of the ovary, where the germarium is located (Figures 3a and b). Oocytes in the oviduct go through vitellogenesis. Acidophilic and heavily stained with hematoxylin, the oogonia and primary oocytes are both oocytes. Oogonia develop deeper pinkish with hematoxylin and eosin stain as vitellogenesis progresses and becomes more basophilic. Figures 3c–g show how tightly packed the yolk granules and globules are inside the mature egg. For this species, there are no oocytes in the posterior portion of the oviduct. The posterior section of this species' oviducts is of simple and unaltered construction because the *C. furcatus* releases fertilised eggs straight into the medium. The length and width of a mature oocyte in this species are 112µm and 70µm, respectively.

Anatomy of the Male Reproductive System

A single genital duct and the median testis make up the male reproductive system. The testis is an organ that is long and narrow at the back. From the anterolateral portion of the testis, the vas deferens emerge and travel through the left side of the perivisceral cavity to the last thoracic segment. The mature spermatozoa are subsequently stored in a sizeable seminal vesicle that develops as it ascends towards the body's centre. The ductus ejaculations in the vaginal segment connect the seminal vesicle to the tubular spermatophore sac, which then opens to the outside (Figure 4).

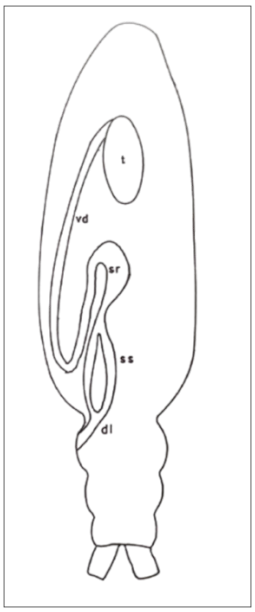
Histology of the Male Reproductive System

The male reproductive system of *C. furcatus* shares several characteristics with *Pseudodiaptomus serricaudatus* and other calanoid copepods in histology. However, the testis and several genital duct locations are very noticeable in this species. The germinal zone's location and the various stages of spermatogenic cells are similar to those of other calanoid copepods, although there are more spermatozoa in the testis' anterior cavity. Other calanoid copepods' spermatozoa are smaller than those of *C. furcatus.* The vas deferens, and seminal vesicle walls are glandular and secrete much material. A substantial amount of core secretion and numerous spermatozoa are tightly packed together in the spermatophore, possibly to retain and nourish the spermatozoa (Figures 5b–l). High fecundity is observed in *C. furcatus* due to the direct broadcasting of fertilised eggs to the medium. As a result, more spermatozoa must be readily available to fertilise the eggs.



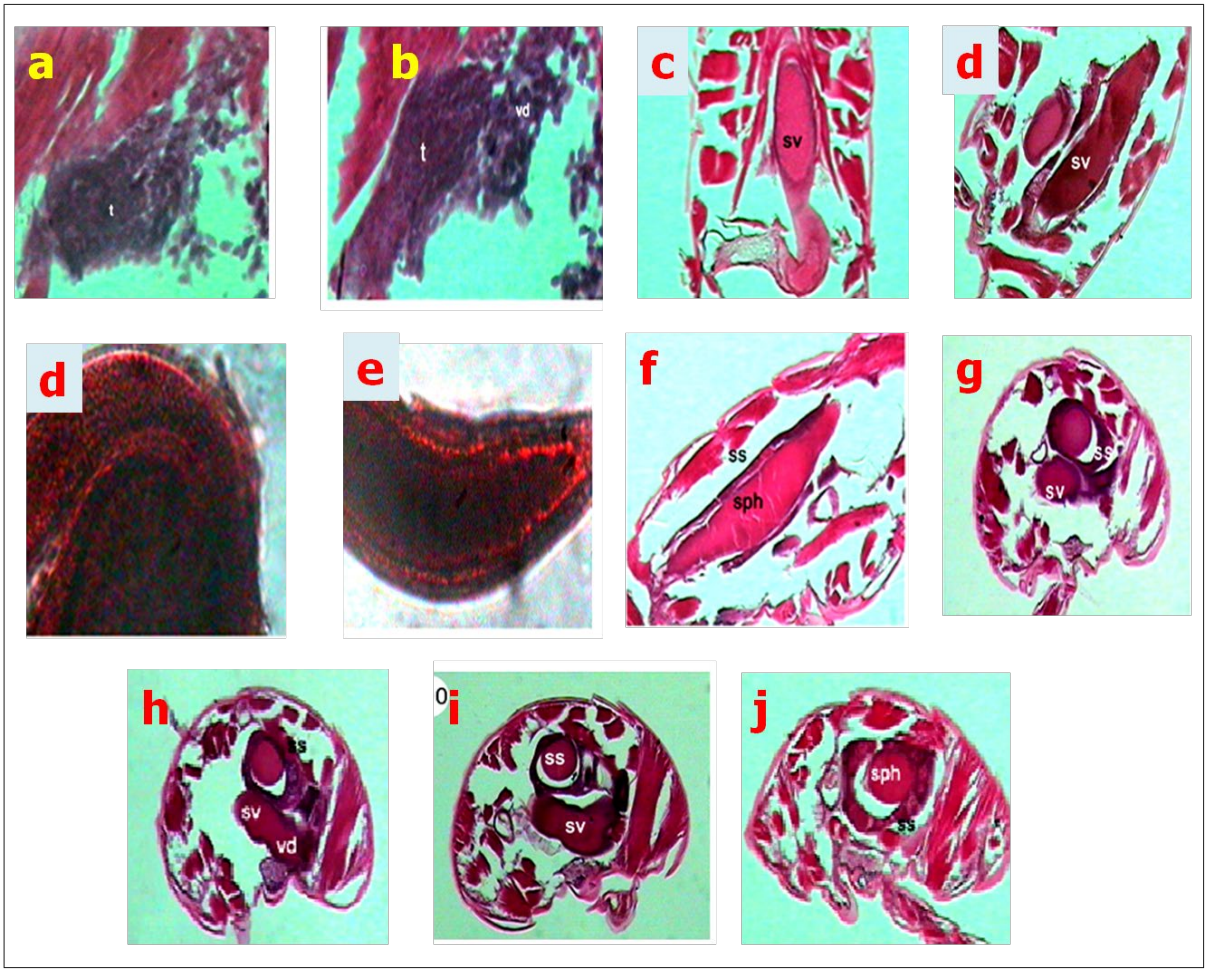
(poc – primary oocytes; voc – vitellogenic oocytes; o – ovary; od – oviduct; odd – oviduct diverticulum; og – oogonial cells; gc – germinal cells)

Figure 3. a and b. Ovary; c and d. Oocytes in the oviduct; e - g. Oocytes in different stages of vitellogenesis



(*t– testis, vs-vas deferens, sv- seminal vesicle, ss- spermatophore sac, de- ductus ejaculations*)

Figure 4. Male Reproductive System



(t – testis; vd- vas deferens; sv – seminal vesicle; ss - spermatophore sac; de - ductus ejaculatoris; sph - spermatophore)

Figure 5. a. Testis; b. Vas deferens; c and d. Seminal vesicle; e and f. Proximal and Distal regions of seminal vesicle; g. Spermatophore sac; h, i and j. C.S. of male showing Vas deferens, Seminal vesicle, Spermatophore sac and Spermatophore

Spermatogenesis and Spermatophore Formation

The spermatogonial cells, which are round and have a prominent, intensely pigmented nucleus, are produced by the testis' germinal cells. The spermatogonial cells change into primary spermatocytes when their volume doubles, subsequently changing into secondary spermatocytes. The smaller secondary spermatocytes, which exhibit a dense nucleus than the earlier stages of spermatogenesis, are reduced to generate the spermatids. The cytoplasm and nucleus of the spermatids alter as they develop into spermatozoa. The nucleus and cytoplasm of the spermatozoa produced by *C. furcatus* are spherical.

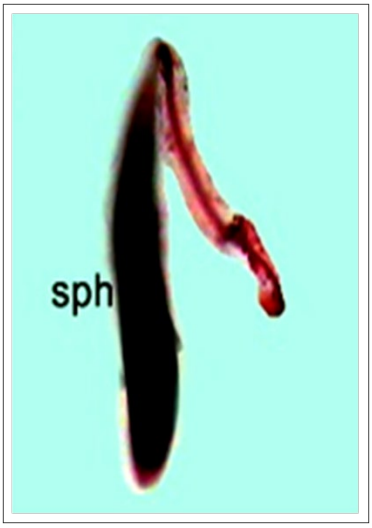


Figure 6: Spermatophore

The secretory material generated by the various genital duct regions is used in the spermatophore sac to form the *C. furcatus* spermatophore. With a broad posterior and a very narrow anterior section, it exhibits an extended tube-like form (Figure 6). During this process, Spermatozoa are moved from the testis to the vas deferens and suspended in the secretory substance. The distal portion of the vas deferens secretes an extra granular secretory material that surrounds the spermatozoa and core secretory material. A very pronounced and lengthy seminal vesicle receives the spermatozoa and the vas deferens secretary material. The spermatophore wall is formed by the secretary material produced by the thick glandular wall of the seminal vesicle. The seminal vesicle includes various spermatophore constituents organised in a spermatophore-like structure. The seminal vesicle's spermatophores material, intended to create a single spermatophore, is released into the spermatophore sac. A tubular spermatophore is created from the spermatophore sac's secretory material, and the spermatophore material is derived from the seminal vesicle. A layer of spermatozoa, an inner layer of secretary material, a layer of core secretary material, and an outside formidable wall make up its structure. Additional secretary material is also produced by the spermatophore sac's wall and functions as an adhesive to secure the spermatophore to the female genitalia. This species has a high reproductive potential and the ability to produce numerous spermatophores, as evidenced by the dense spermatogenic cells in the testis, the highly glandular form of the vas deferens and seminal vesicle, and the vast quantity of spermatozoa, and secretary material in the genital duct. Under laboratory conditions, an adult *C. furcatus* showed a life span of 38 days and produced four batches of eggs (28ºC temperature, 30 PSU salinity, pH 7.8 and dissolved oxygen 5.5 mg/L). The average egg measures 74 ±3 µm in diameter, and 92 ±14 eggs were counted in a batch. This species has six nauplii and six copepodite stages, with a post-embryonic development period of seven (7 ±1) days.

The body length of different naupliar stages and the prosome length of copepodite stages of *C. furcatus* is presented in Table 1. The length of the nauplii and copepodite stages of *C. furcatus* was found to be size-wise suitable live prey for fry to fingerling stages of marine finfish larvae.

Table 1. Body length and prosome length of naupliar and copepodite stages of *C. furcatus* (µm)

	Body Length (µm)		Prosome Length (µm)
Nauplii	NI 108 ±3	Copepodite	CI 326 ±11
	NII 122 ±5		CII 378 ±14
	NIII 167 ±4		CIII 590 ±18
	NIV 212 ±12		CIV 810 ±23
	NV 247 ±6		CV M 1210 ±21
	NVI 281 ±9		CV F 1340 ±17
	--		CVI M 1482 ±26
	--		CVI F 1638 ±18

The calanoid copepods that are free-living exhibit either perennial or seasonal occurrences. While *Centropages congeners* showed a seasonal succession, species like *Acartia clausi*, *Temora longicornis*, and *Pseudocalanus* spp. were reported throughout the year. According to Razouls et al. (2005–2023), the *C. furcatus* is a widely distributed epipelagic species that primarily inhabits equatorial and subtropical regions. According to Kavitha et al. (2018), the East coast of India experiences many *C. furcatus* sightings throughout the year. Most copepod species' yearly reproductive cycles exhibited similar patterns, with maxima in egg production rates occurring between the end of April and June and substantially lower rates occurring in the other months. However, for species like *Centropages typicus*, the highest egg production rates were seen towards the middle of September, during the first week of the species' appearance in the plankton (Halsband and Hirche, 2001). Although many calanoid species from cooler climates have been shown to have a comparable reproductive cycle and egg production (Lindley 1990), species from warmer climates are continuous breeders and actively reproduce all year long (Ianora and Scotto di Carlo, 1988; Ianora and Buttino, 1990). Compared to *Pseudodiaptomus* species from India's east coast, *C. furcatus* has more documented fecundity (Altaff, 2020). Many calanoid copepod species' egg production is significantly impacted by environmental conditions such as temperature, salinity, and phytoplankton content (Dilshad Begum et al., 2912; Dvoretsky and Dvoretsky, 2014). The calanoid copepods exhibited apparent sexual dimorphism, as evidenced by the larger size of the female, the geniculated antennule of the male, the presence of more urosomal somites in the male, the different pattern of the caudal setae in the female, and the modified P5 in both sexes. The male's antennule is asymmetrical and extensively adapted to facilitate copulation and spermatophore transfer to the female during mating, in contrast to the female's simple and symmetrical antennule. Both sexes'

fifth thoracic legs frequently differ from the typical morphology of the other thoracic legs. In the female, they may be diminished or completely absent. Still, in the male, they are typically changed to form a complex prehensile organ that allows them to grasp the female and attach spermatophores to the female vaginal pore. *P. annandalei*, *P. serricaudatus*, and *T. discaudata* had more modified right antennules in the male and P5 in both sexes than *C. furcatus* (Altaff, 2020).

In most calanoid copepod species, the female reproductive system is characterised by a median ovary, two oviducts, and a segment of the genitalia containing the seminal receptacle (Marshall and Orr, 1972; Corkett and McLaren, 1978; Blades-Eckelbarger and Youngbluth, 1984; Razouls et al., 1986, 1987; Norrbin, 1994; Eisfeld and Niehoff, 2007). The size and shape of the ovary, the diverticula in the oviduct's posterior area, the structure of the antrum, and the structure of the seminal receptacles, on the other hand, are all subject to modification (Altaff, 2020). Compared to other calanoids, the female reproductive system of C. furcatus demonstrates greater specialisation, with a conspicuously large ovary and oviducts occupying the anterior to the posterior portion of the prosome. Such large oviducts and their diverticular suggest the ability to generate more eggs.

According to Eckelbarger and Blades-Eckelbarger (2005), the ovarian histology of *C. furcatus* is consistent with the pear-shaped structure previously described for calanoid copepods. Calanoid copepods typically have ovaries in the middle of the prosome, and several stages of ovarian cells arranged spatially from the posterior to the anterior. Similar arrangements are seen in *C. furcatus*, where vitellogenesis and oocyte maturation occurs outside the ovary while the mitotic proliferation of germinal cells and their transformation into previtellogenic oocytes stage through the meiotic process take place inside the ovary. The most mature oocytes form the most ventral layer as some calanoid copepods' previtellogenic oocytes migrate from dorsal to ventral throughout the anterior and posterior diverticula (Niehoff, 2007). However, Ceballos-Vazquez et al. (2009) showed a significant difference regarding the oocyte migration from the ovary in *C. furcatus*. The current study shows this difference in oocyte migration from the ovary to the oviducts and diverticular.

Previtellogenic oocytes in *C. furcatus* were discovered to disperse from the ovary and arrange themselves on the inner wall, forming an outer layer that spans the prosome's entire length and circumference, allowing for a greater area for oocyte maturation and, as a result, favouring continuous egg production. The present study demonstrates such a configuration of vitellogenic oocytes and the vitellogenesis and oocyte maturation processes. Niehoff (2007) classified the *Centropages* species. *C. bradyi, C. hamatus, C. typicus,* and *C. violaceus* as having gonads of the Calanus type, indicating that most oocyte development stages take place simultaneously and that many oocytes typically mature synchronously. The geographical distribution of oocytes is an essential trait that differs from those of the Calanus-type gonad (Ceballos-Vazquez et al. 2009). According to studies done on Calanus-type gonads (Niehoff & Hirche, 1996), the most mature oocytes form the most ventral layer and expand and mature to varying degrees along the lengths of both anterior and posterior diverticula (Niehoff, 2007). The most mature oocytes are found closest to the middle of the body in *C. furcatus,* where the increase in oocyte size and degree of maturation was observed to occur radially towards the centre. From there, the oocytes are carried ventrally through the products to the genital pore. Based on these observations, Ceballos-Vazquez et al. (2009) proposed the furcatus-type gonad as a new gonad morphological type for *C. furcatus.* The current findings support Ceballos-Vazquez et al. (2009)'s hypothesis regarding the kind of *C. furcatus* gonad shape and ongoing egg-laying.

Regarding spawning and embryonic development, calanoid copepods primarily use two primary tactics. In certain species, eggs are released into the environment after fertilisation, and embryonic development occurs in the external medium. In other species, eggs are released into the ovisac following fertilisation. The posterior portion of the oviduct changes into a glandular structure for ovisac production, producing secretory material specifically for ovisac formation. Unlike species whose embryonic development occurs in the ovisac, the *C. furcatus* belongs to the broadcasting form of spawning, which allows for more significant egg production. For *C. typicus*, Carlotti et al. (2007) found that the combination of a temperature-dependent development rate and a food-dependent growth rate in the shelf regions is a more favourable environment than in the offshore regions, as the shelf regions support large females, the production of large numbers of better-quality eggs, and probably better offspring survival. For the *C. furcatus*, a similar observation is applicable.

The male is an active partner in finding and capturing the female in the reproductive biology of calanoid copepods. The right antennule in the male and P5 in both sexes, which play numerous vital roles during copulation and spermatophore transfer, are sexually dimorphic appendages that have evolved. The male first recognises a mate; then, the female is captured. The male transfers and attaches a spermatophore to the female removes the discharged spermatophore, and finally, the female fertilises the eggs and releases them (Ohtsuka and Huys, 2001). *C. furcatus* has also been found to exhibit such sophisticated sexual dimorphism (Maria et al.,

2013). For calculating the male-to-female ratio in the mass culture of this species, *C. furcatus* has an advantageously quick and easy copulation and spermatophore transfer process.

The germinal zone is in the posterior portion of the testis of C. furcatus, similar to the case of other calanoid copepods. According to Marshall and Orr (1972), Park (1966), Corkette and McLaren (1978), Hopkins (1978), and others, all species' germinal cells are spherical in shape, extensively stained with haematoxylin, and have giant nuclei. Nevertheless, there is variety in their size. As the various phases of spermatogenesis occur in ascending rows from the posterior to anterior region, the process of spermatogenesis is completed within the testis in all species (Hopkins, 1978). This spermatogenetic process has been observed in the majority of calanoid copepods. Although the histology of the testis of *C. furcatus* is like that of other calanoid copepods, in this species, the testis is ovoid in shape during the CIV and CV stages and becomes elongated in the CVI stage. The posterior part of the testis contains germinal cells, and other spermatogenic stages occupy the anterior part of the testis in ascending rows. This species' spermatozoa have a spherical form and are not mobile.

The modification of the *C. furcatus* genital duct into the vas deference, seminal vesicle, spermatophore sac, and ductus ejaculators show similarity to other calanoid copepods (Blades-Eckelbarger and Youngbluth, 1991a). All of these regions of the genital duct in *C. furcatus* are extensively developed with ample secretory material. Typically, calanoid copepods use the secretory material produced by the vas deference, seminal vesicle, and spermatophore sac to produce an elongated or flask-shaped spermatophore in the spermatophore sac. However, the variety of secretory materials generated depends on the sort of spermatophore a calanoid species produces. According to Park (1966), Hopkins (1978), Blades and Youngbluth (1981), and Blades-Eckelbarger (1991), the front section of the spermatophore of calanoid copepods can either be a simple tube or a sophisticated coupling plate-like structure. When mating and spermatophore transfer, the spermatophore of *C. furcatus* is of a fundamental kind with a narrow and long tube that may be linked to the female's genital hole simply and precisely. This promotes excellent reproductive potential.

The current study on the *C. furcatus* reproductive system revealed several distinctive characteristics, including year-round natural occurrence, continuous breeding, and high reproductive capacity in both sexes. The high fecundity of this species, which produces more than 100 eggs in each clutch, is also far higher than the fecundity reported for many calanoid copepods that are now being mass-produced. Numerous marine finfish larvae in various developmental stages can be found as live prey on *C. furcatus* nauplii, copepodites, and adults in the abovementioned size range. This species' broadcasting form of spawning also allows mass production of diapausing dry eggs. These qualities make *C. furcatus* a potential top choice for mass cultivation and use in aqua hatcheries to develop finfish larvae.

Conclusions

An Indigenous live feed is essential for rearing marine finfish larvae in hatcheries to enhance their growth and survival rates. Copepods serve as suitable live prey, and the commercial production and utilisation of copepods could have beneficial mariculture. Compared to the free-living planktonic marine calanoid copepod species occurring in nature, only a small number have been mass-cultured and utilised as live prey for rearing marine finfish larvae. The present study reports the reproductive biology of a potential calanoid copepod, *C. furcatus,* and its advantageous features for mass production.

Compliance with Ethical Standards

Conflict of interest: The authors declare that they have no actual, potential, or perceived conflict of interest for this article.

Ethics committee approval: Ethics committee approval is not required for this study.

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Coccidian infestation in cultured common pandora (*Pagellus erythrinus*)

Tülay AKAYLI, Çiğdem ÜRKÜ

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Istanbul University, Faculty of Aquatic Sciences, Department of Aquaculture and Fish Diseases, Istanbul, Turkiye

ORCID IDs of the author(s):
T.A. 0000-0003-2375-2224
Ç.Ü. 0000-0003-0381-9321

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Correspondence:
Çiğdem ÜRKÜ
E-mail: yavuz. curku@istanbul.edu.tr

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ABSTRACT

Coccidia is a spore-forming obligate intracellular protozoan parasite that causes disease in many fish species. This study aimed to diagnose a parasitic disease case that affected a common pandora (*Pagellus erythrinus*) with a high mortality rate. The samples prepared from the internal organs of the diseased fish and the gills and muscle tissues were examined parasitologically using histological methods, a light microscope, and Transmission Electron Microscopy (TEM). No parasites or parasitic formations were found in the wet mount preparations. The presence of parasitic spores (1-1,5 x 0.3-0.7 µm) was detected towards the intestinal tissue between the intestinal microvilli of fish in the electron microscopy study. On the other hand, histological examination showed that a cystic structure full of spores (sporocyst) 550-750 µm in size developed in the abdominal muscles of the infected fish. At the same time, there were no such structures in the intestines. As a result, since the parasite spores observed in the diseased fish are very small, they settle in the cell and pass through the intestines by forming cysts in the abdominal muscles. They were identified as Coccidian *sp.* because of their similar morphology to those of the parasites in the Coccidian group. Coccidian infestation was detected in this fish species for the first time in this study. However, detecting the spores' entrance through the fish's intestines in the early stage and observing a small number of sporocyst structures suggest that the disease is in the development stage.

Keywords: Fish, *Pagellus erythrinus,* Parasite, Coccidian, TEM, Histology

Introduction

Common pandora (*Pagellus erythrinus*), belonging to the Sparidae family, is a valuable species for mariculture in the Mediterranean region (Le Breton, 1999; Klaoudatos et al., 2004). Fish are successfully produced at the hatching stage in some marine aquaculture enterprises on Turkey's Aegean coastline. In the last decade, the worldwide aquaculture production of common pandora has progressively dropped from 197.00 tonnes to 0.04 (FAO, 2021). Therefore, more studies are needed to improve the production of this species in offshore net cage systems. To achieve this successful aquaculture production, it is necessary to take into account some important disadvantages such as low growth performances, decreased quality of flesh, increased ratio of mortality, susceptibility to diseases, and skin colouration properties that do not match the wild specimens (Divanach et al., 1993; Başçınar, 2004; Cascarano et al., 2021).

The Apicomplexa phylum consists of unicellular and mostly parasitic sporozoan parasites. Coccidian is spore-forming obligate intracellular protozoan parasites, which are members of the Apicomplexa group and are usually found in the intestinal tissues of freshwater and marine fish (Steinhagen et al., 1990; Molnar, 2006; Shrestha, 2022). This parasite group has a complex life cycle that includes different stages (sporogony, merogony and gamogony) and produces resistant sporocysts in different parts of organs and tissue within a single host (Dyková and Lom, 1981; Harding and Frischknecht, 2020; Shrestha, 2022). However, piscine coccidia is intracellular organisms of epithelial tissues (intestine, gallbladder, swim bladder, kidney tubules and liver) according to their developmental sites and life cycles (Molnar, 2006; Sitjà-Bobadilla et al., 2016).

Coccidiosis in cultured fish is usually a chronic infection with gradual mortality. These parasites live intracellularly in the intestines of fish, adversely affecting the fish's immune system. They cause direct death, slow development, high sensitivity to opportunistic pathogens and low resistance to stress (Molnar, 2006; Sitjà-Bobadilla et al., 2016; Shrestha, 2022). The main endoparasitic protozoans that cause coccidiosis in cultured gilt-head sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) belong to *Eimeria, Goussia* and *Cryptosporidium* species. (Sitjà-Bobadilla and Alvarez-Pellitero, 2002; Gjurčević et al., 2017; Fioravanti et al., 2020). In Türkiye, *Eimeria merlangi* (Özer et al., 2012) and *E. sardinae* species have been reported in the intestines of whiting fish (*Merlangius merlangus*) caught by fishing (Özer et al., 2014).

There are structurally diverse life cycle stages and host-parasite interfaces in fish coccidian (Molnar, 2006; Sitjà-Bobadilla et al., 2016; Fioravanti et al., 2020). Scientists are investigating in detail the complex structures of this type of parasite formed during their intracellular development and the mechanism of the spores, which are the perfect invasion machines of Apicomplexans, with the electron microscope (Steinhagen et al., 1990; Molnar, 2006; Dogga et al., 2015; Sitjà-Bobadilla et al., 2016). Identifying coccidian infestation at the early stage is very important for preventing and treating the disease. Nevertheless, there is limited information about the mechanisms of transportation of the parasite's early stages (Steinhagen et al., 1990; Molnar, 2006; Cascarano et al., 2021; Shrestha, 2022).

The current study aimed to investigate the presence of intracellular spore-formed protozoan parasites that affect the gut and muscle tissues of naturally infected cultured common pandora (*Pagellus erythrinus*) by electron microscopy and histological methods.

Materials and Methods

Sampling

Moribund common pandora (BW 45-100 *g*) were detected on the surface of the cages at the aquaculture farm located on the Türkiye Aegean Sea coast, which recorded 45% mortality. Samples for bacteriology, histopathology and electron microscopy were taken from six diseased fish.

Bacteriology

The internal organs (spleen, liver and kidney) of the fish samples (n=6) were inoculated into Trypticase Soy agar (TSA) containing 1.5% NaCl. Routine bacteriological laboratory methods and an API 20E rapid diagnosis kit were used to determine the biochemical properties of isolated bacteria.

Electron Microscopy

The gut tissue samples (n=6) were prepared for transmission electron microscopy (TEM). Prefixation of the gut samples was carried out in 3% glutaraldehyde solution buffered with 0.1 M sodium cacodylate, and they were post-fixed in 2% OsO_4 and dehydrated in a series of ethanol treatments. Afterwards, the gut samples were passed through propylene oxide and embedded in an open. Ultrathin sections were cut, double-stained using uranyl acetate (Watson, 1958) and Reynold's lead citrate (Reynolds, 1963), and examined under an electron microscope (TEM).

Histology

Processing of tissue samples from the liver, kidney, spleen, gills, gut, and abdominal muscle for histopathology was performed after they were fixed in 10% buffered formalin. After the routine processing of the tissues for histology was performed, 5 μm sections prepared from paraffin blocks were stained with haematoxylin and eosin (H&E) (Bruno et al., 2006).

Results and Discussion

Clinical Findings

In the moribund fish samples, haemorrhages on the pectoral fins and supraorbital region and swelling in the abdominal region drew attention externally (Figure 1a). Adiposity in internal organs and paleness in the liver colour were observed internally (Figure 1b).

Bacteriological Findings

In the general bacterial examination, no bacterial colonies were isolated from the inoculation made from most fish samples. Solely, *Aeromonas* spp. were recovered and identified from the liver of only one fish sample.

Electron Microscope Findings

In the detailed examination performed with transmission electron microscopy (TEM), 3 oval-shaped parasitic spores trying to migrate to the host were found between the microvilli in the hindgut of the diseased fish (Figure 2a). It was noted that the average size of these intracellular spores was between 1-1,5 x 0.3- 0.7 μm and that they headed for the intestinal tissue of the host. The oval-shaped parasitic and pyriform-like infective spores reaching the intestinal tissue were detected in another tissue section (Figure 2b). This infective sporoplasm increasing in size was observed trying to migrate to the host cell. In Figure 2c, it was observed that an active infective spore penetrated the intestinal epithelial cell and tried to extrude its content into the cytoplasm of the host cell. In the same example, a parasitic spore, which completed this process, lost its structure, and started to melt, was detected.

By the data obtained from the electron microscope examination, because parasitic spores in the intestinal sections of diseased fish were very small and they tried to migrate to the body of the host through the intestinal tract, they were determined to show similarities to the general characteristics of the obligate parasites in the *Coccidian* group (Pasnik et al., 2005).

Therefore, these intracellular parasite spores were identified as *Coccidian* sp.

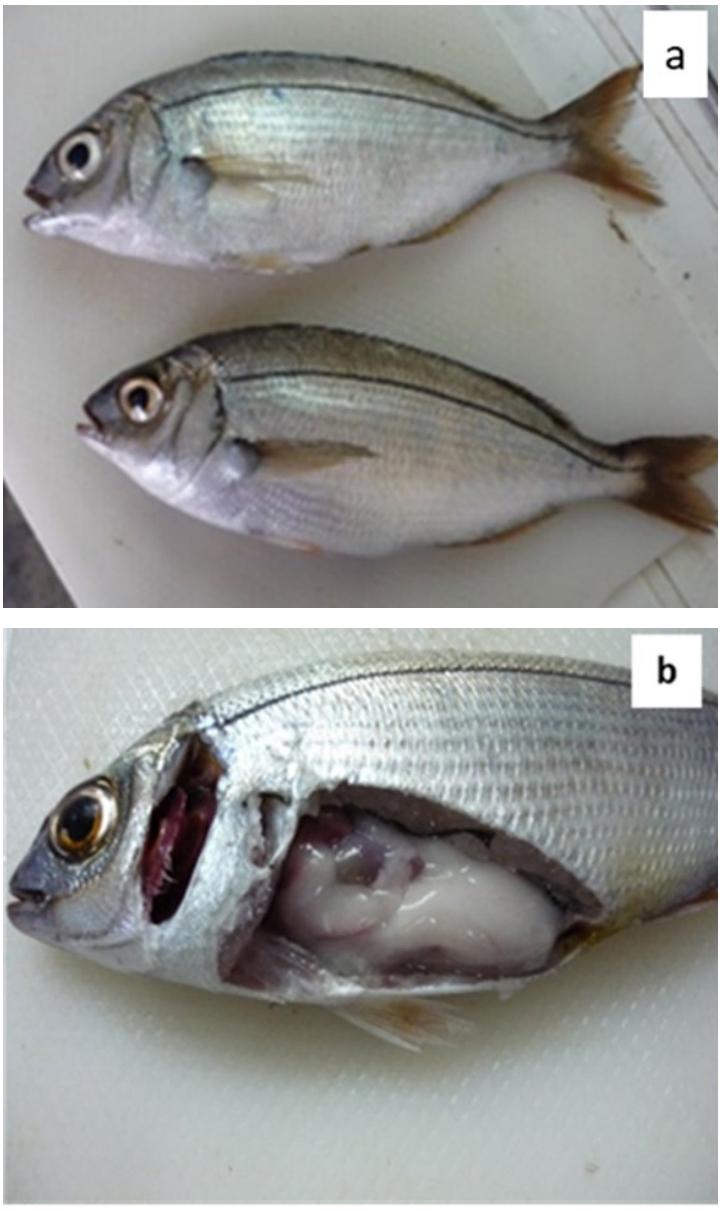

Figure 1. External (a) and internal (b) view of diseased fish

Histological Findings

No parasite or parasitic formations were detected in the histopathological examination of various internal organs such as the kidney, liver, intestine, spleen, and gills of diseased fish. However, while the general histologic appearance of the intestinal tissue of the examined fish was normal (Figure 3a), the presence of vacuolar degeneration and diffuse hemorrhagic foci within the tissue was noted in the detailed examination with a higher magnification (Figure 3b).

Moreover, it was observed that fish had intense tissue loss in the abdominal muscle tissue, and the amount of adipose tissue

increased significantly due to necrosis. Furthermore, a sporocystic structure in the ellipsoidal sporophorous vesicle, 550-750 µm in size, was noted in this region (Figure 4a). Numerous parasitic spores ranging between 1.5 x 20 µm in size were observed in the cyst (Figure 4b).

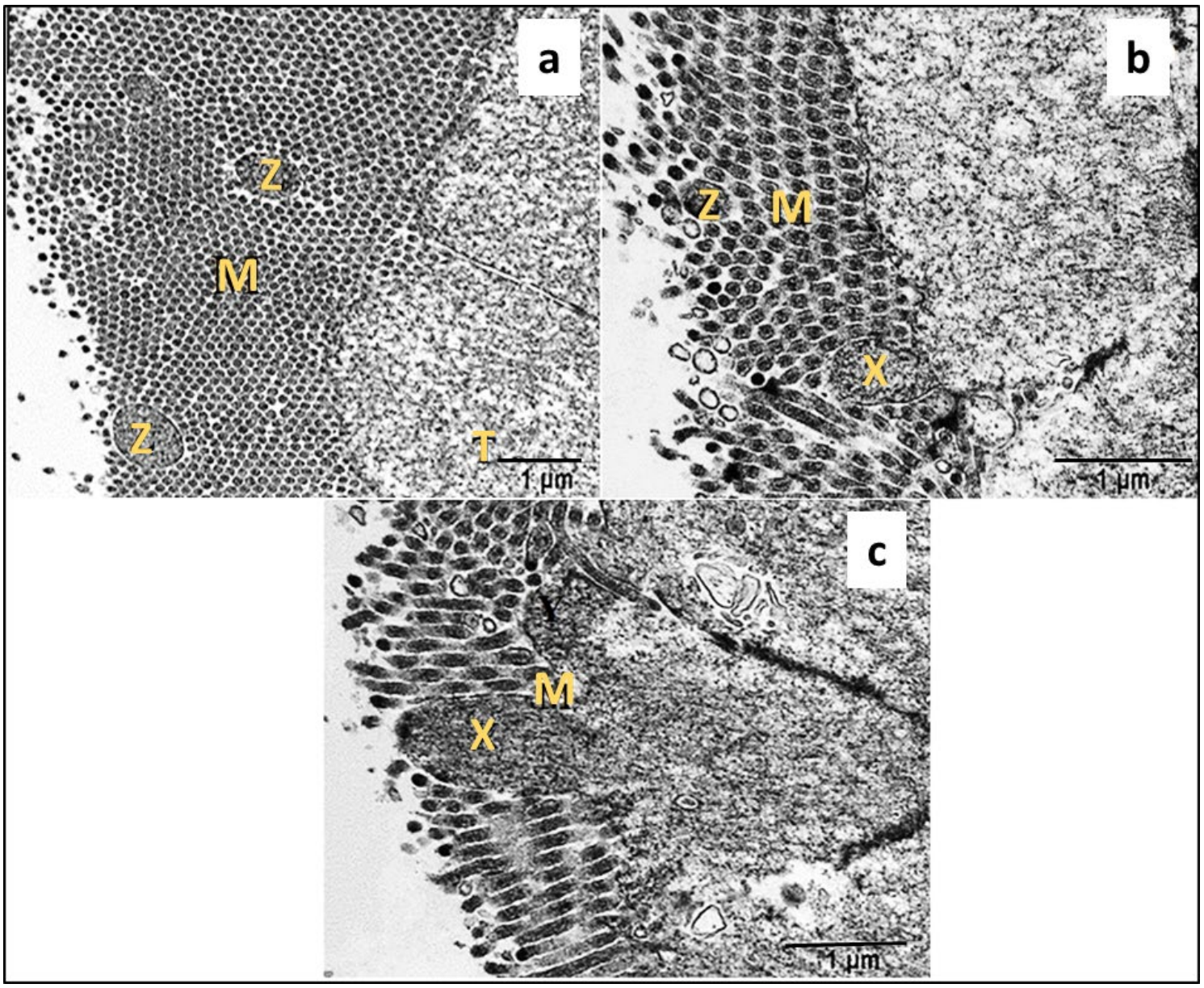

Figure 2 (a, b, c). The appearance of motile parasite spores between the transverse and longitudinal sections of the intestinal villi of fish (TEM). (**M:** microvillus, **Z:** parasitic spore, **X:** pyriform-like infective spores, **Y:** the appearance of spore entering the tissue, **T:** intestinal epithelial cell membrane)

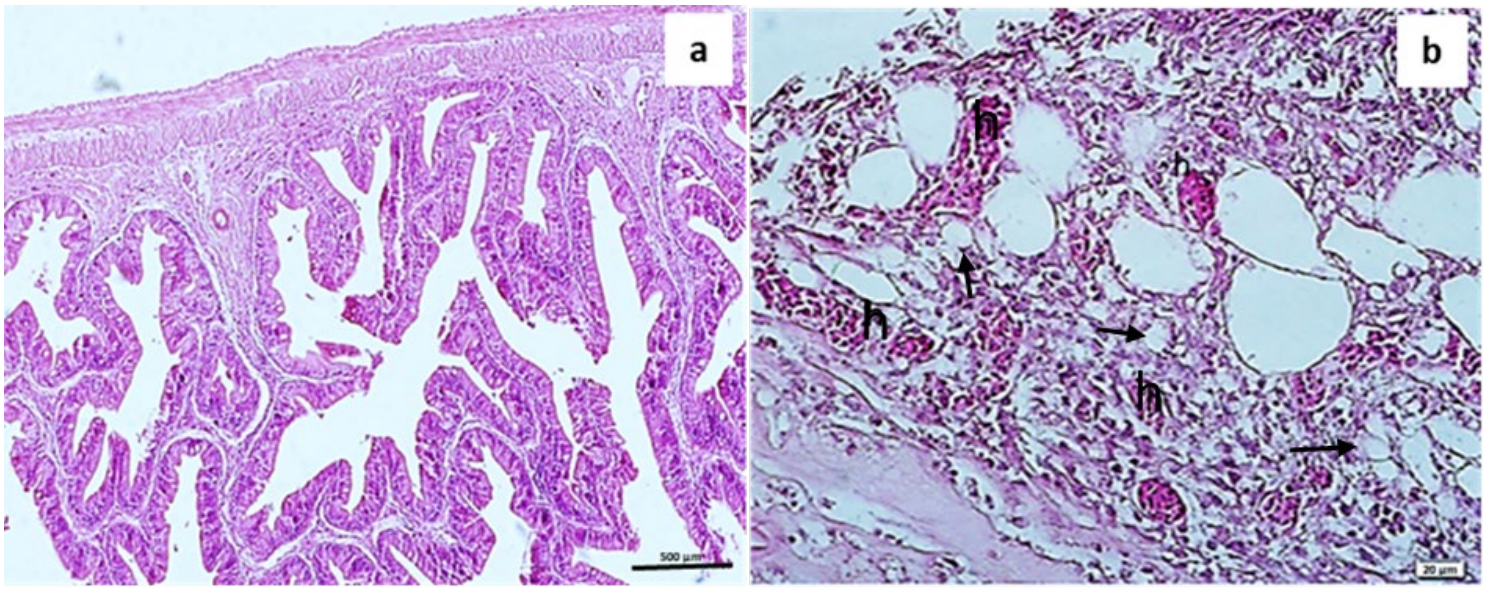

Figure 3. (a) General view of intestinal tissue of fish, **(b)** vacuolar degeneration (arrowed) and diffuse hemorrhagic foci (h) within the tissue

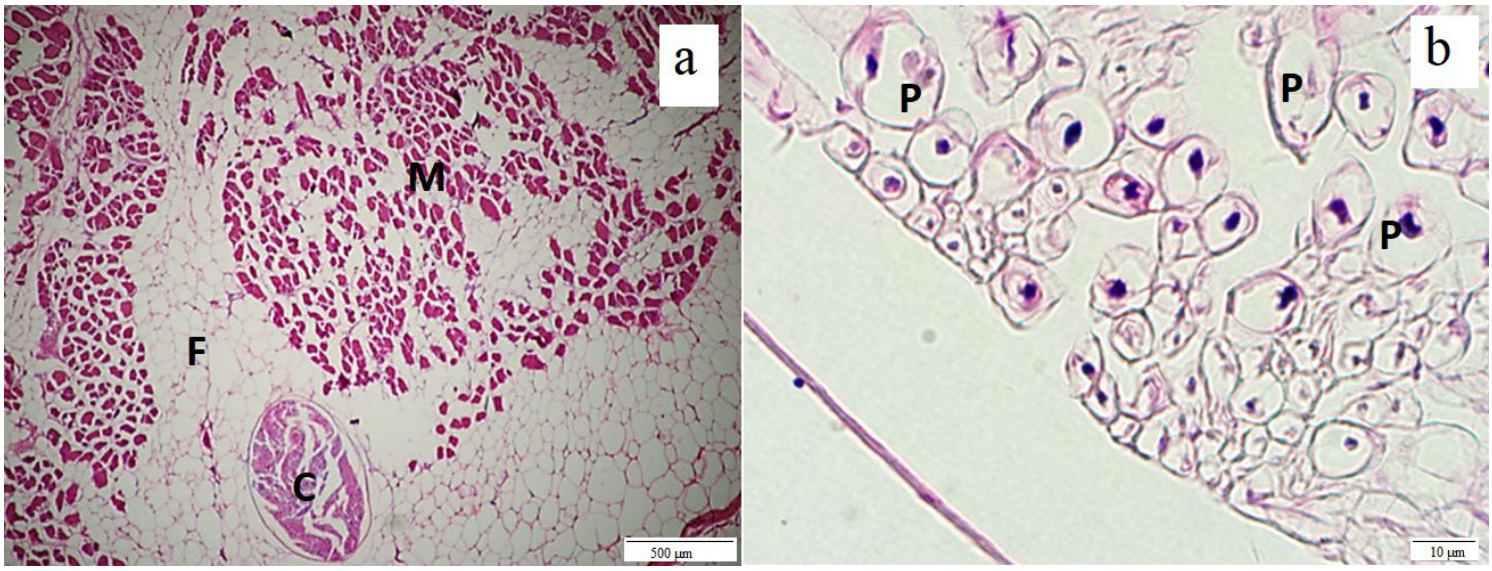

Figure 4. (a) General view of the sporocystic structure (C) in the abdominal muscle tissue of the diseased common pandora, (**b**) Appearance of numerous parasitic spores inside the cyst (C). (**M:** muscle, **F**: fatty tissue, **C**: sporocyst, **P:** parasitic spore)

As a result of the histological examination, it was concluded that the sporocystic structure containing numerous spores in the muscle tissue of diseased fish is similar to the sporocystic formations caused by the coccidian parasites in the muscle (Gjurčević et al., 2017).

Knowing the early stages and host penetration mechanisms of coccidian parasites is very important for the progression of infestations (Steinhagen et al., 1990; Molnar, 2006; Cascarano et al., 2021; Shrestha, 2022). Consequently, the growth rate of cultured fish reduces; which negatively affects the productivity of fish farms by causing high mortality in infected fish in advanced stages (Alvarez-Pellitero, 2004; Fioravanti et al., 2020; Shrestha, 2022).

Coccidia that is pathogenic to marine and freshwater fish do not have a distinct locomotion organelle, but their motile spores move forward with gliding movement and then enter the living cell with its apical complex (apicoplast) (Dyková

and Lom, 1981; Harding and Frischknecht, 2020). The researchers examined the ultrastructural features of the apical complex and spores using electron microscopy to identify the parasites. Apicoplast was found to be present in the genera *Eimeria*, *Goussi*, *Toxoplasma* and *Sarcocystis* but not in *Cryptosporidium* (Molnar, 2006; Harding and Frischknecht, 2020). It has been reported that species such as *Eimeria sparis*, *E. dicentrari, E. bouixi*, *Goussia sparis* and *Cryptosporidium molnari* were found in the intestinal tissue of gilthead sea bream and European sea bass cultured in Mediterranean countries and caused infestation (Alvarez-Pellitero, 1995; Gjurčević et al., 2017; Fioravanti et al., 2020).

Coccidian parasites have a complex life cycle with three primary stages: sporogony, merogony and gamogony. The sporogony phase occurs in the fish's external environment when the sporozoites enter the fish's gut. Coocidian parasite spores that enter from the intestines of fish transform into trophozoites in a vacuole structure that surrounds them in the intestinal tissue at the next stage. These cells become self-similar and multinucleated schizonts after the schizogony (asexual multiple division) stage. The cells developing in the schizont turn into an orange slice-shaped merozoite, and the merogony stage begins (Steinhagen et al., 1990; Dogga et al., 2015, Shrestha, 2022). In an experimental *Cryptosporidium molnari* infestation in gilt-head sea bream, all three stages were observed in different internal organs (Sitjà-Bobadilla and Alvarez-Pellitero 2002; Sitjà-Bobadilla et al., 2016). Yang et al. (2016) reported that *C. molnari* in koi fish (*Cyprinus carpio*) also causes severe granulomatous inflammatory lesions in the gills, liver, spleen, kidneys, and intestines of the fish. The size of coccidian sporozoites causing infection in fish is approximately between 0,5-1.5 µm x 20 µm (Sitjà-Bobadilla et al., 2016; Gjurčević et al., 2017; Harding and Frischknecht; 2020). This study determined that the parasitic spores of 0,3-1,5 x 0.5-30 µm in size enter the intestine. Infective parasitic spores passed through the microvilli, developed in this stage, and reached the intestinal cell by penetrating the host cell membrane. The patterns exhibited by the spores detected in the fish samples are very similar to the life cycle of coccidian parasites (Bruno et al., 2006; Harding and Frischknecht, 2020).

The present study determined using electron microscopy and histological examination techniques that parasite spores passed from the intestine to the fish, and cysts formed (550-750 µm in size) in the muscle tissue in the next stage. In the cyst, numerous parasitic spores ranged between 3.5 x 5 µm in size. Moreover, the studies performed with the electron microscope revealed that coccidian parasite spores migrate to the host cell through the intestinal microvilli. In contrast, the histological examination of the tissue sections revealed that the spores progress towards the abdominal muscles and form a sporocystic structure. As indicated by other researchers, the present electron microscope study revealed that the parasitic spores, which are found in the surrounding aquatic environment of the diseased common pandora and enter through the anus, pass through the intestines and reach the fish via anal gavage (Steinhagen et al., 1990; Molnar, 2006; Dogga et al., 2015; Sitjà-Bobadilla et al., 2016). The variation of the parasite localisation among *Eimeria*, *Goussia* and *Cryptosporidum* species made identifying this coccidian species difficult. The cystic structure was not observed in the intestinal tissue of the infected fish, but local hemorrhagic foci and vacuolisation were noted. However, the cystic structure developing in the muscle tissue containing many nuclei and oval-shaped spores indicates that it is in the schizont-forming stage (Steinhagen et al., 1990; Dogga et al., 2015; Shrestha, 2022).

Conclusion

Light and electron microscopy and histological techniques are widely preferred to describe coccidian parasites' taxonomy, life cycle and developmental forming. The very small and oval-shaped parasite spores in the intestines of the diseased common pandora (*Pagellus erythrinus*), which formed sporocysts in the abdominal muscles, were identified as *Coccidian* sp. However, since the ultrastructural properties and histological findings in the current investigation covered only some of the life stages of the sporozoan parasite in detail, a complete identification could not be made. We can only suggest that the intracellular parasite has a low capability to spread infection in the host cell and therefore develops at the onset of the disease. These findings will help expand our understanding of coccidian-induced diseases of the fish.

Compliance with Ethical Standards

Conflict of interest: The authors declare that they have no actual, potential, or perceived conflict of interest for this article.

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Do the length-weight relationships and condition factors of farmed rainbow trout, brook, and brown trout differ from their wild counterparts?

Ömerhan DÜRRANİ

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Karadeniz Technical University, Institute of Marine Sciences and Technology, Trabzon 61080 Türkiye

ORCID IDs of the author(s):
Ö.D. 0000-0003-1775-8662

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Correspondence:
Ömerhan DÜRRANİ
E-mail: ukhan@ktu.edu.tr

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ABSTRACT

This study examines the length-weight relationships (LWR) and condition factors (CF) of three farmed fish species: rainbow trout (*Oncorhynchus mykiss*), brook trout (*Salvelinus fontinalis*), and brown trout (*Salmo trutta*). It then compares these findings with existing literature data for their wild counterparts to gain insights into the influence of aquaculture on their growth patterns. Using a simple power function, $W = \alpha L_T^{\beta}$ where W represents the fish's weight, and L_T represents the fish's total length, the LWR is determined. The estimated β values indicate positive allometric growth for rainbow and brook trout, whereas brown trout exhibit an isometric growth pattern. The estimated condition factors ranged from 0.992 to 1.442 for rainbow trout, 0.665 to 1.731 for brook trout, and 0.841 to 1.321 for brown trout, with significant differences observed among them (Kruskal-Wallis test, $p < 0.05$). Compared with literature data from their wild counterparts, notable variations in growth patterns emerge, particularly evident in rainbow and brook trout, possibly illustrating the contrasting effects of aquaculture.

Keywords: Aquaculture, Freshwater, Length-weight relationship, Wild-caught fish, Salmonidae

Introduction

Aquaculture significantly contributes to the global food supply, with 90.86 million tonnes of aquatic animals valued at USD ~275.54 billion in 2021, marking a ~57.31% increase from 2010 (FAO, 2022; FishStatJ, 2023). Ultimately, this industry plays a crucial role in reducing overfishing of wild fish by providing a sustainable alternative source of seafood, meeting the rising demand for protein and essential nutrients inherent to aquatic animals (Ye & Beddington, 1996; Lem et al., 2014; Kobayashi et al., 2015; Babu & Joshi, 2019).

Farm-raised fish, which are cultivated in controlled pens within lakes, oceans, or rivers, as well as fish raised in large tanks, can exhibit notable differences in characteristics compared with their wild counterparts caught from their natural habitats (Johnston et al., 2006; Gaviglio & Demartini, 2009; Molversmyr et al., 2022). These differences include various characteristics such as carcass composition, taste profile, texture, and overall quality. Generally, these differences arise from the prevailing trend in fish farming, centred on cost reduction and enhanced productivity through genetic advancements and the formulation of specialised diets (Gjedrem, 1997; Quinton et al., 2005; Johnston et al., 2006). Consequently, farmed fish typically tend to have a more significant proportion of muscle mass and fat content in their carcasses, resulting from controlled feeding practices and optimised growth conditions in aquaculture settings (Laird, 1997; Johnston et al., 2006; Başçinar et al., 2007; Deng et al., 2016). In addition to these, significant morphological differences have been identified between wild and farmed fish across various fish species (Von Cramon-Taubadel et al., 2005; Jawad et al., 2020). Their length-weight relationships (LWR) have shown considerable variation, enabling the distinction between wild and farmed fish (Naeem et al., 2011; Hassan, 2021).

Escapees from farmed fish have significant implications, affecting the aquaculture industry and the surrounding wild populations (Arechavala-Lopez et al., 2013). These escapees can have detrimental effects on the wild ecosystem. For example, they can prey on native species, compete for vital resources such as food availability, territorial space, and suitable breeding habitats, potentially spread parasites and diseases, and even interbreed with wild fish (Jonsson & Jonsson, 2006; Grigorakis & Rigos, 2011; Atalah & Sanchez-Jerez, 2020). Apart from the several negative consequences of farmed escapees, a significant issue arises when farm-aggregated wild fish are occasionally caught from the wild and fraudulently mislabelled as genuine "wild fish" (Bell et al., 2003; Morrison et al., 2007). This deceptive practice directly impacts the assurance of fish quality for consumers, eroding their trust in the market (Arechavala-Lopez et al., 2013). Several methods, such as genetics, chemical analysis, fatty acid composition, trace elements, stable isotopes, pollutants, morphology, and sensory characteristics, have been used to identify and distinguish escapees of farmed fish from their wild counterparts (Arechavala-Lopez et al., 2013). There is an obvious necessity to prioritise the development of cost-effective tools, such as morphometric methods, for effectively detecting farmed individuals within the wild population (Arechavala-Lopez et al., 2013; Dürrani et al., 2023).

The objective of this study was to examine the LWR and condition factors (CF) of farmed rainbow trout (Oncorhynchus mykiss), brook trout (Salvelinus fontinalis), and brown trout (Salmo trutta). These measurements were then compared with the existing literature data of their wild counterparts. This study should provide essential baseline data that can aid in identifying and distinguishing escapees of farmed fish from their wild counterparts in the natural environment.

Materials and Methods

Fishes Acquisition

The trout hatchery at the Sürmene Faculty of Marine Sciences, KTÜ, Çamburnu, provided the farmed specimens of rainbow trout, brook trout, and brown trout. In the hatchery, these fish were fed commercial diets acquired from Skretting Aquaculture, a subsidiary of Nutreco based in Türkiye. The commercial feed contained ~44% crude protein and ~21% crude fat for larger fish, whereas smaller fish were fed diets comprising approximately ~55% crude protein and ~12% crude fat. The hatchery receives freshwater from a nearby brook and continuous aeration in each fish tank to maintain optimal oxygen levels. The annual water temperature fluctuates between 7° C and 22° C throughout the year.

Length-Weight Relationship

The fish's total lengths (LT) were measured to the nearest 0.1 cm, and their body weight (W) was measured to 0.01 g for each species. The length-weight relationships were determined by the simple power function (Basusta & Dürrani, 2021):

$$W = \alpha L_T^{\beta} \quad (1)$$

Where α represents the intercept and β represents the slope.

An estimated value of β equal to 3 signifies the isometric growth of fish. If β is less than 3, fish exhibit negative allometric growth, becoming slimmer as their length increases. If β

is greater than 3, fish display positive allometric growth, becoming heavier and reflecting optimal growth conditions (Mazlum & Turan, 2018). The statistical deviation of β from the hypothetical value of 3.0 (within the isometric range) was tested using Student's t-test to evaluate isometry.

Condition Factor

The condition factor (CF) was calculated using the following function (Bal, 2021):

$$C_{\mathrm{F}} = \frac{W \cdot 100}{{L_{\mathrm{T}}}^3} \quad (2)$$

The non-parametric Kruskal-Wallis test was used to assess significant differences in CF of different farmed fishes due to the non-normal distribution of Cf data. Significant differences were considered when $P < 0.05$.

Results and Discussion

Length-Weight Relationship

The minimum and maximum lengths of rainbow trout ranged from 15.2 to 33.2 cm, brook trout ranged from 13.4 to 32.4 cm, and brown trout ranged from 14.0 to 33.0 cm (Table 1).

The estimated values with 95% confidence intervals (CI) for β were 3.10 ±0.10 for rainbow trout, 3.55 ±0.15 for brook trout, and 3.10 ±0.16 for brown trout (Table 2). The Student's t-test analysis revealed significant deviations in the β values of rainbow trout and brook trout from the isometric range of 3.0, indicating a positive allometric growth pattern in these fish species. Conversely, the estimated β value of brown trout exhibited no significant deviation from the isometric range, suggesting an isometric growth pattern for this fish.

Table 1. Body measurements of three farmed fish species acquired from a local fish hatchery in Trabzon, Türkiye

Fish species	*n*	Estimated ± 95% CI	
		Total length (cm)	Total weight (g)
Rainbow trout	157	23.777 ±0.759	182.505 ±15.788
Brook trout	160	23.036 ±0.892	185.46 ±20.725
Brown trout	93	22.902 ±1.468	169.304 ±26.474

The parameterised simple power function $W = \alpha {L_{\mathrm{T}}}^{\beta}$ for each fish species was used to provide the curve lines in Figure 1, which illustrate the relationship between LT and W for rainbow trout, brook trout and brown trout.

Condition Factor

The estimated minimum and maximum CF for rainbow trout was 0.992–1.442, for brook trout 0.665–1.731, and brown trout 0.841–1.321. No differences in CF were found between rainbow trout and brook trout, but both differed significantly from brown trout (Figure 2).

Table 2. Summary statistics of the length-weight relationships $W = \alpha {L_{\mathrm{T}}}^{\beta *}$, along with the Student's t-test to evaluate the deviation of the estimated β value from the isometric range

Fish species	Length-weight relationships			Student's *t-test* for *β*		Growth pattern
	α	*β*	Adj. R^2	*t*	*p*-value	
Rainbow trout	0.009 ±0.003	3.101 ±0.096	0.979	2.055	0.041	Positive allometric
Brook trout	0.002 ±0.001	3.554 ±0.146	0.969	7.445	0.000	Positive allometric
Brown trout	0.008 ±0.005	3.095 ±0.164	0.984	1.135	0.258	Isometrics

*W, total weight of fish (g), LT, total length (cm)

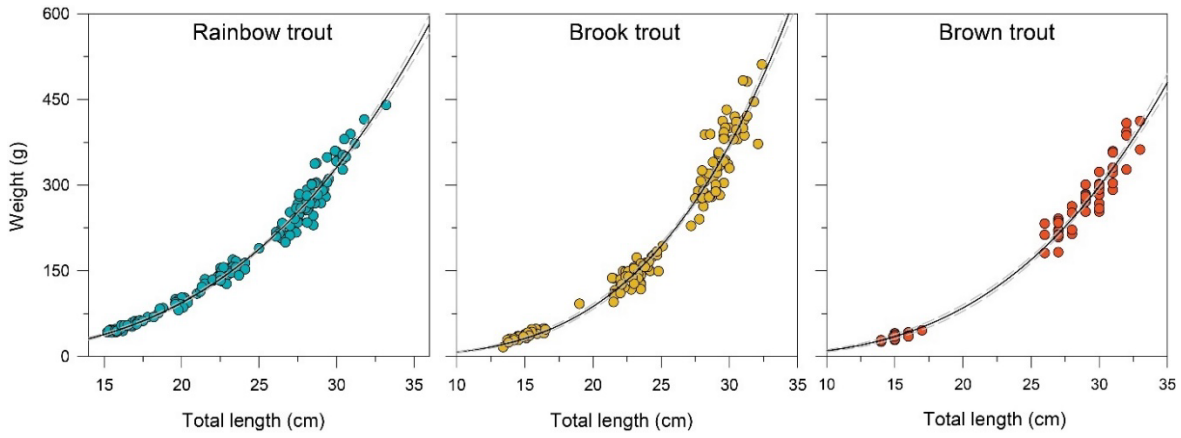

Figure 1. Length-weight relationships for three farmed fish species as determined by $W = \alpha L_T{}^{\beta}$, where W represents the total weight of the fish (g) and L_T represents the total length (cm). The solid lines represent the fitted model, and the dashed lines represent the 95% confidence interval. The coefficients for each species were as follows: rainbow trout: $\alpha = 0.009$, $\beta = 3.101$, brook trout: $\alpha = 0.002$, $\beta = 3.554$, and brown trout: $\alpha = 0.008$, $\beta = 3.095$.

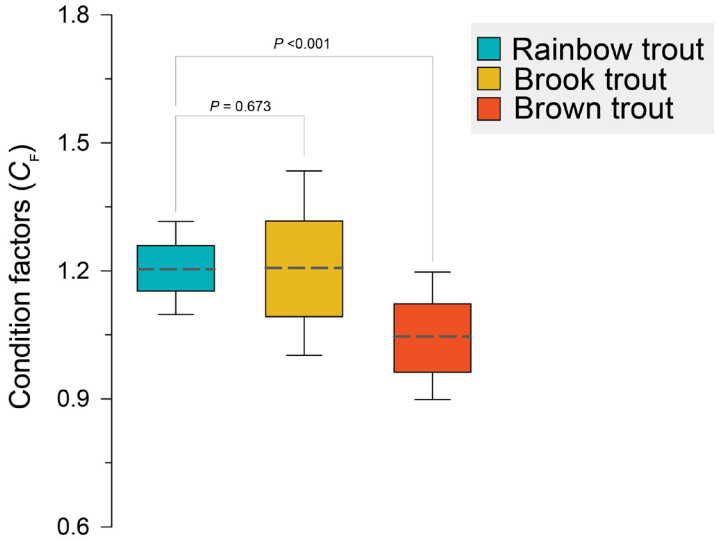

Figure 2. Boxplots of condition factor (C_F) for farmed fish species acquired from a local fish hatchery in Trabzon, Türkiye. Dashed lines indicate the mean values. Significant differences in C_f were checked with the Kruskal-Wallis test, followed by Dunn's post hoc test.

This study demonstrated positive allometric growth of rainbow trout, which aligns with the findings of Ahmad and Ahmed (2019), who determined β of 3.393 in October and 3.384 in December. On the other hand, Wali et al. (2019) determined β = 3.028, suggesting the isometric growth rate of farmed rainbow trout. Furthermore, this study also demonstrated positive allometric growth in brook trout, which is inconsistent with the results of Onder and Khan (2016). Their study determined isometric growth in monoculture but observed negative allometric growth in duoculture. For brown trout, they reported isometric growth in both culture conditions, which is consistent with the findings of this study. The wild brown trout also showed isometric growth determined by Arslan et al. (2004) and Verreycken et al. (2011). In contrast to the present study, rainbow trout and brook trout in the wild had negative allometric growth patterns with β ranging between 2.604 and 2.843 (McAfee, 1966; Ruiz-Campos et al.,

1997; Adams et al., 2008; Verreycken et al., 2011; Wali et al., 2019; Rios & Teixeira de Mello, 2020).

The condition factor CF is a commonly employed measure for assessing the overall health of fish: a condition factor CF of 1 generally indicates good condition, while <1 suggests slimness in fish, and more than 1 indicates fatness of fish (Piper, 1972; Joergensen, 2017). In this study, the CF of brown trout was 1.038, significantly smaller than that of rainbow trout and brook trout. In the wild, all these fishes have relatively lower CF, e.g., 0.97 for rainbow trout and 1.05 for brook trout, as Bravo et al. (2021) reported. Likewise, LWR, the farmed and wild brown trout tend to have similar values of CF (1.04), as reported by Bravo et al. (2021). According to Piper (1972), the CF of salmonids typically remains constant as long as there is consistency in water temperature and the feeding rate. Thus, the variation in CF among fish can be attributed to various factors, including food availability and environmental conditions, which significantly impact the overall health of the fish (Luther, 1963). The seasonal CF differences result from varying feeding intensity and reproductive changes (Ahmad & Ahmed, 2019).

Conclusion

Providing appropriate feeding and water conditions in aquaculture promotes positive allometric growth in farmed rainbow and brook trout. As a result, farmed fish exhibit an immense body depth compared with their wild counterparts. In contrast, the negative allometric growth observed in wild populations of these species may indicate challenges related to food availability and environmental conditions. However, unlike rainbow trout and brook trout, the LWR and CF of brown trout in aquaculture are similar to their wild counterparts. Further studies are needed to investigate the impact of different feeding regimes and environmental conditions on the LWR and CF of farmed fishes, which will help identify the primary factors influencing fish allometry. The results of such studies can provide valuable baseline data for distinguishing escaped farmed fish from their wild counterparts in the natural environment.

Compliance with Ethical Standards

Conflict of interest: The author declares that they have no actual, potential, or perceived conflict of interest for this article.

Ethics committee approval: The numerical data used in this study were collected from harvested fish without any welfare concerns, thereby preventing the need for Ethics Committee Permission since no laboratory experiments were involved.

Data availability: Data will be made available on request.

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Phytoplankton communities of two floodplain lakes of the Dibru Saikhowa biosphere reserve, Tinsukia, Assam (Northeast India): Ecology, richness, and abundance

Nogen NOROH[1], **Kensibo PAMAI**[2], **Mrinal HATIMURIA**[3]

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[1] 6 No., Shantipur, Sadiya, Tinsukia District, Pin-786157, Assam, India

[2] Patkai Christian College (Autonomous), Department of Zoology, Chümoukedima-Seithekema, Pin-797103, Chümoukedima, Nagaland

[3] Dr. Radhakrishnan School of Arts, Commerce & Science, Dibrugarh, Pin- 786001, Assam, India

ORCID IDs of the author(s):

N.N. 0009-0000-0057-2517

K.P. 0009-0008-7411-6221

M.H. 0009-0007-3044-9756

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Correspondence:

Kensibo PAMAI

E-mail: kensibo971@gmail.com

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ABSTRACT

Phytoplankton communities of Dibru Saikhowa Biosphere Reserve (DSBR) beels were studied from October 2013 to September 2015 in two floodplain lakes (beels), namely Maghuri beel and No.11 beel in Tinsukia district, upper Assam, Northeast India. Phytoplankton reveal a richness of (61 species) belonging to five groups: Chlorophyta (35 species) > Bacillariophyta (13 species) > Euglenophyta (7 species) > Cyanophyta (5 species) > Dinophyta (1). The monthly phytoplankton richness indicated 13–32 (25 ±6) species) and 21–39 (30 ±5) and with distinct species importance of Chlorophyta (5-17) 12 ±4 and (10-24) 15 ±3 species in Maghuri beel and No.11 beel respectively. Phytoplankton abundance ranged between 162 ±157 n/L and 138 ±39 n/L and comprised a sub-dominant component of net plankton, i.e., between 39.7 ±15.8% and 41.0 ±9.9% in Maghuri beel and No.11 beel respectively. Seventeen abiotic factors recorded relatively limited influence on the phytoplankton richness and abundance of the sampled bells. The canonical correspondence analysis asserted higher cumulative influence along the first two axes of 17 abiotic factors on phytoplankton assemblages of Maghuri beel (76.46%) than in No.11 beel (61.73%) beels.

Keywords: Beels, Conservation area, Composition, Distribution, Phytoplankton, Chlorophyta

Introduction

The floodplain lakes are unique ecosystems supporting aquatic life forms of diverse plants and animals and are considered the most critical and productive ecosystem. The floodplain is ideal for limnological considerations vis-à-vis aquatic biodiversity, water quality, ecology, and biological productivity. Little is known about phytoplankton richness, abundance ecology and their role in biological productivity in these environs of India (Jana, 1998). The earlier studies from northeastern India are confined to preliminary reports by Sharma (2004), who initiated a detailed analysis of phytoplankton of a floodplain lake of upper Assam. Sharma (2009) studied phytoplankton's composition, abundance, and ecology in Loktak Lake (a Ramsar site), Manipur. This study is based on the detailed analysis of phytoplankton assemblages of the selected floodplain lakes (beels) in upper Assam. The investigations merit ecosystem diversity, biogeography and ecological importance for Indian limnology and phytoplankton biodiversity in wetlands of conservation areas of India in particular.

Materials and Methods

Limnological studies were undertaken for two years monthly from October 2013 – September 2015, in two floodplain lakes (beels) named Maghuri (27° 34' 19.2" - 27° 34' 25.2" N; 95° 22' 04.5"-95° 22' 35.2" E; altitude: 96.1 m ASL; area: 1197 ha) and No. 11 (27° 34' 04.8"-27° 34' 11.5"N; 95° 20' 21.8"-95° 20' 25.8" E; 94.7 m ASL; area: 12 ha) beels located in the 'buffer zone' of the Dibru-Saikhowa Biosphere Reserve (DSBR), Tinsukia district, upper Assam. The sampled beels are invariably referred to as 'DSBR beels' in this article.

Aquatic vegetation of these beels included *Eichhornia crassipes, Pistia stratiotes*, *Lemna* sp., *Azolla* sp., *Ludwigia* sp., *Rumex* sp. *Cabomba caroliniana*, *Hygroryza aristata*, *Trapa natans*, *Eleocharis* sp., and *Nymphaea* sp.

Water temperature, pH and specific conductivity were recorded with the help of field probes and dissolved oxygen was estimated by the modified Winkler's method. The other abiotic parameters, such as free carbon dioxide, total alkalinity, total hardness, calcium, magnesium, chloride, dissolved organic matter, total dissolved solids, phosphate, nitrate, sulphate, and silica, were analysed following APHA (1992). The rainfall data was obtained from the Citrus Research Station, Government of Assam, Tinsukia, Assam.

The qualitative plankton samples were collected by towing nylobolt plankton net (No. #50 µm), and the quantitative samples were by filtering 25 litres of water from the selected sites at regular monthly intervals and were preserved in 5% formalin. Various phytoplankton taxa were screened with a Wild Stereoscopic Binocular Microscope for isolation and were observed with a Leica (DM 1000) stereoscopic phase contrast microscope fitted with an image analyser. The phytoplankton taxa were identified following the works of Tiffany and Britton (1952), Needham and Needham (1962), Islam and Haroon (1980), Adoni et al. (1985), Fitter and Manuel (1986) and Perumal and Anand (2008), and several research papers.

The percentage similarities (Sorenson's index), Species diversity (Shannon's index), Dominance (Berger-Parker's index), and Evenness (Pielou's index) were calculated following Ludwig and Reynolds (1988) and Magurran (1988). Ecological relationships between the abiotic and biotic parameters were determined by Pearson correlation coefficients (r_1 and r_2, respectively, for Maghuri beel and No.11 beel). The canonical correspondence analysis (XLSTAT 2014) was done to observe the cumulative influence of stated abiotic factors on phytoplankton assemblages.

Results and Discussion

The observed variations in abiotic factors (mean ± SD) of two regularly sampled beels, namely the Maghuri beel and No.11 beel, are indicated in Table 1. Water temperature corroborated with the geographical location of the wetlands. Specific Conductivity showed low ionic concentrations and, thus, warranted the inclusion of DSBR beels under the 'Class 1' category of trophic classification vide Talling and Talling (1965). Slightly acidic to alkaline nature of waters and soft to moderate waters of these beels depict moderate dissolved oxygen and free carbon dioxide, low concentration of micro-nutrients and other abiotic factors. The Chloride concentrations in the beels registered low and thus indicated a lack of influence of organic pollution caused by human impact.

Richness

Sixty-one species of phytoplankton belonging to five groups: Chlorophyta (35 species) > Bacillariophyta (13 species) > Euglenophyta (7 species) > Cyanophyta (5 species) > Dinophyta (1) were documented from DSBR beels. The temporal variation of phytoplankton between the sites is indicated in Table 2. Maghuri beel and No.11 beel recorded species richness of 61 species each. The phytoplankton richness concurred with the 62, 61 and 59 species recorded from Utra and Waithou pats (Sharma, 2010) of Manipur and Deepor beel (Sharma, 2015) of Assam while it showed a more diverse nature than the earlier reports from 49 and 55 species from Rawalsar and Prashar lakes of Himachal Pradesh (Thakur et al., 2013); 52

species from Samuajan beel (Sharma, 2004) and Ghorajan beel (Sharma, 2012) from Assam, respectively. However, the phytoplankton richness of DSBR beels is lower than 75 species reported from Loktak Lake (Sharma, 2009), Manipur. Chlorophyta (35 species) depicted qualitative importance of phytoplankton are characterised by the rich desmid genera, namely *Cosmarium* (6 species) = *Micrasterias* (6 species) > *Staurastrum* (4 species) > *Closterium* (3 species) = *Euastrum* (3 species) > *Pediastrum* (2 species) which collectively comprised ~51.0 % of the Chlorophyta richness in the sampled beels. Desmid diversity is an essential indicator of waters with low ionic concentrations and Calcium content (Payne, 1986; Sharma, 1995; Sharma and Pachuau, 2016). This important characteristic is attributed to the salient features of the water quality of DSBR beels.

Bacillariophyta (13 species) recorded importance but showed lower richness than the reports of Sharma (2004, 2009, 2010, 2012, 2015) and Khan (2017). The monthly phytoplankton richness ranged between 13–32 (25 ±6) species) and 21–39 (30 ±5) species in the Maghuri beel and No.11 beel during the study period. It did not show any significant correlation with abiotic factors during the study period, thus indicating a lack of the role of abiotic factors vis-à-vis phytoplankton diversity. The present study showed no definite periodicity of the richness of phytoplankton in the sampled beels concurrent with the remarks of Sharma (2004, 2010, 2012, 2015) in certain floodplain lakes of NEI and from other water bodies of Meghalaya (Sharma and Lyngskor, 2003; Sharma and Lyngdoh, 2003) and Mizoram (Pachuau, 2009).

The phytoplankton community similarities ranged between 14.6–77.2 % in the Maghuri beel and 36.0–74.7 % in the No.11 beel, respectively. The recorded ranges suggested heterogeneity in phytoplankton composition in DSBR beels during the study period. The heterogeneity remarks are endorsed by the facts that the similarity matrices indicated lower similarity, i.e., 31-40%, 41-50% and 51-60% in 49, 75 and 92 instances (~78% of total instances), respectively in Maghuri beel; and 41-50% and 51-60% in 79 and 124 instances (~74% of total instances) in No.11 beel. The hierarchical cluster analysis endorsed heterogeneity in phytoplankton assembles of two beels during the study.

Abundance

Phytoplankton is characterised by low abundance, i.e., between 162 ±157 n/L in Maghuri beel and 138 ±39 n/L in No. 11 beel (Noroh, 2019); it comprised a sub-dominant component of net plankton, i.e., between 39.7 ±15.8% and 41.0±9.9% in Maghuri beel and No.11 beel respectively during the study period. Phytoplankton recorded relatively more comprehensive density variations in Maghuri beel and contributed significantly to quantitative variations of net plankton in Maghuri (r_1= 0.974, p< 0.0001). The abundance broadly concurred with the reports from Nigeen Lake, Kashmir Himalayas (Shafi *et al*., 2013) and certain beels of lower Assam (Khan, 2017) while it is lower than the results from floodplain lakes (Sharma, 2010) of Manipur; Deepor Beel (Sharma, 2015), Samuajan beel (Sharma, 2004) and Ghorajan beel (Sharma, 2012) of Assam; and the Majuli floodplains lakes (Hatimuria, 2015).

Phytoplankton abundance did not follow any definite fluctuation pattern during the study period. The former generalisation concurred with the reports of Sharma (2010, 2012). Still, it differed from the trimodal pattern observed in Loktak Lake (Sharma, 2009) and Deepor beel (Sharma, 2015) and also from bimodal variations reported by Yadava *et al.* (1987), Sanjer and Sharma (1995) and Jindal et al., (2014). Chlorophyta > Bacillariophyta recorded phytoplankton dominance in No.11 beels during the study period but showed Bacillariophyta > Chlorophyta during the first year in Maghuri beel thus indicating a little deviation in quantitative importance. Cyanophyta and Euglenophyta exerted limited importance in the selected beels. The stated variations are attributed to ecological heterogeneity amongst DSBR beels. The significance of Chlorophyta concurred with the reports from specific aquatic ecosystems of northeast India (Goswami and Goswami, 2001; Sharma, 2009, 2010, 2012, 2015; Hatimuria, 2015), while Bacillariophyta importance in Maghuri beel concurred with the reports of Baruah *et al.* (1993). Chlorophyta comprised an important component (45.6 ±15.6% and 47.8 ±7.6%) and contributed notably (r_1= 0.711, p= 0.0001) and (r_2= 0.894, p< 0.0001) to quantitative variations of phytoplankton of Maghuri beel and No.11 beel, respectively during the study period. Peak density of Chlorophyta was recorded during February 2014 in the Maghuri beel and March 2014 in the No.11 beel. Chlorophyta indicated relatively lower abundance with the reports of Sharma (2004, 2009, 2010, 2015) from the floodplain lakes of northeast India as well as from certain reservoirs of Meghalaya (Sharma, 1995; Sharma and Lyngdoh, 2003; Sharma and Lyngskor, 2003).

Chlorophyta is characterised by the quantitative importance of certain desmid taxa, namely *Cosmarium* spp. (9 ±9 n/L, 11 ±7 n/L) and *Closterium* spp. (15 ±11 n/L, 13 ±7 n/L) and limited importance of *Micrasterias* spp. (5 ±4 n/L, 11 ±9 n/L) during the study period in Maghuri beel and No.11 beel, respectively. The present result of the quantitative role agreed with the importance of certain species of green algae indicated by Sharma (2004). Bacillariophyta, the second most diverse group after Chlorophyta, showed abundance ranged between 16.7-80.4% (41.3±20.1)% and 18.0-44.8% (32.2±6.9)

% in Maghuri beel, and No.11 beel respectively during the study period. Bacillariophyta abundance did not follow any definite pattern of variation throughout the study period, which, in turn, contrasts the results of Sharma (2012) and differs from a trimodal pattern reported by Deepor beel (Sharma, 2015). Annual maxima were observed in March 2014 and May 2015 in both Maghuri beel and No.11 beel, respectively. The Diatom abundance lacked the distinct role of any individual species, as reported by Sharma (2015).

Species Diversity, Evenness and Dominance

The species diversity of phytoplankton is influenced by richness and equitability, or relative abundance of species, and it is recorded in the following stated order of species diversity (*vide* Shannon's index) of phytoplankton of No. 11 beel (2.812-3.401, 3.133 ±0.165) > Maghuri (1.161-3.012, 2.570 ±0.446) beel. The characteristic differences are further endorsed by higher diversity (> 3.0) during 19 months in No.11 beels, while such a condition is noticed during eight and one months in the Maghuri beel. The results thus endorsed phytoplankton heterogeneity on account of habitat diversity and ecological differences amongst the beels. Chlorophyta richness contributed significantly to phytoplankton richness (r_1= 0.830, p< 0.0001 and r_2= 0.845, p <0.0001) in Maghuri beel and No.11 beel, respectively. It is influenced by richness and equitability or relative abundance of species. The phytoplankton diversity did not show any definite pattern of variation during the study period. The most diverse and species-rich Chlorophyta > Bacillariophyta contributed to the phytoplankton diversity in the sampled beels.

Phytoplankton dominance is characterised by consistently low values in the No.11 beel (0.0690.180, 0.116 ±0.031) but indicated certain variations in the Maghuri beel (0.104-0.776, 0.255 ±0.161). In general, low phytoplankton dominance in the sampled beels is attributed to low abundance and equitable distribution of different species (Osborne *et al.,*1976), while selected instances of high dominance resulted from the quantitative importance of fewer phytoplankton species (Whittaker, 1965). The latter conclusion is particularly valid for Maghuri beel during February and March (2014) with the density importance of *Volvox aureus* and *Closterium* spp. (*C. moniliferum*) in particular. The variations of dominance between the beels concurred with the earlier reports from various aquatic ecosystems of NEI (Sharma and Lyngdoh, 2003; Sharma, 2004, 2010, 2012, 2015). Dominance positively correlated with phytoplankton abundance (r_1= 0.835, p= 0.0001), Bacillariophyta abundance (r_1= 0.862, p= 0.0001) and inversely correlated with species diversity (r_1= -0.916, p< 0.0001) in Maghuri beel.

Phytoplankton communities of DSBR beels exhibited moderate to high evenness during the study period, i.e., between 0.335-0.950 (0.813 ±0.142) and 0.884-0.962 (0.930 ±0.018) in Maghuri beel and No.11 beel, respectively. High evenness observed during several months is attributed to the equitable abundance of the majority of phytoplankton taxa (Washington, 1984). Evenness variations concurred with the report from the Majuli floodplains, Assam (Hatimuria, 2015). Phytoplankton evenness is negatively correlated to phytoplankton abundance (r_1= -0.910, p= 0.0001), Bacillariophyta abundance (r_1= -0.907, p= 0.0001), *Volvox aureus* (r_1= -0.695, p= 0.0002) and dominance (r_1= -0.951, p= 0.0001); it is correlated positively with species diversity (r_1= 0.886, p < 0.0001) in Maghuri beel. Phytoplankton evenness was inverse correlated with dominance (r_2 = -0.816, p< 0.0001) in No.11 beel.

Canonical Correspondence Analysis (CCA)

The canonical correspondence analysis asserted higher cumulative influence along the first two axes of 17 abiotic factors on phytoplankton assemblages of Maghuri beel (76.46%) than in No.11 beel (61.73%) beel. CCA coordination biplots indicated the influence of rainfall and dissolved oxygen on net plankton abundance, Chlorophyta richness, and sulphate on phytoplankton density and *Cosmarium* spp. the abundance of dissolved organic matter in Maghuri beel. Net plankton abundance was influenced by chloride, dissolved organic matter and silicate; Chlorophyta abundance and richness were influenced by total alkalinity and plankton richness by total hardness in No.11 beel. The present study recorded limited influence of individual abiotic factors, and CCA results suggested the cumulative importance of seventeen abiotic factors vis-à-vis variations of phytoplankton assemblages DSBR beels.

Ecological Relationships

The present study did not register any significant influence of individual abiotic factors on phytoplankton richness and its constituent group. The results thus depicted a limited role of individual abiotic factors vis-à-vis phytoplankton richness. This conclusion marked a little deviation from the much-limited influence of individual abiotic parameters recorded in certain beels of Assam (Sharma, 2012, 2015) and the importance of certain abiotic factors noted in two floodplain lakes of Manipur (Sharma, 2010).

The phytoplankton abundance (r_1= 0.681, p= 0.0002), Bacillariophyta abundance (r_1= 0.704, p= 0.0001) and *Volvox aureus* abundance (0.632, p= 0.0009) indicated positive correlation with nitrate in Maghuri beel. Cyanophyta abundance (r_2= 0.623, p= 0.0011) positively correlates with rainfall and

Cosmarium spp. abundance is positively correlated with nitrate (r_2 = 0.662, p = 0.0004) in No.11 beel. The stated remarks depicted the role of abiotic factors vis-à-vis phytoplankton concurred with the reports of Sharma and Lyngskor (2003) and Sharma (2004, 2010, 2012). The limited role of abiotic parameters concurred with the results of Sharma (2004, 2012), while it deviated from the influence of some factors indicated by Sharma (2009) or even lack of importance of any individual abiotic parameters as reported by Sharma (2015).

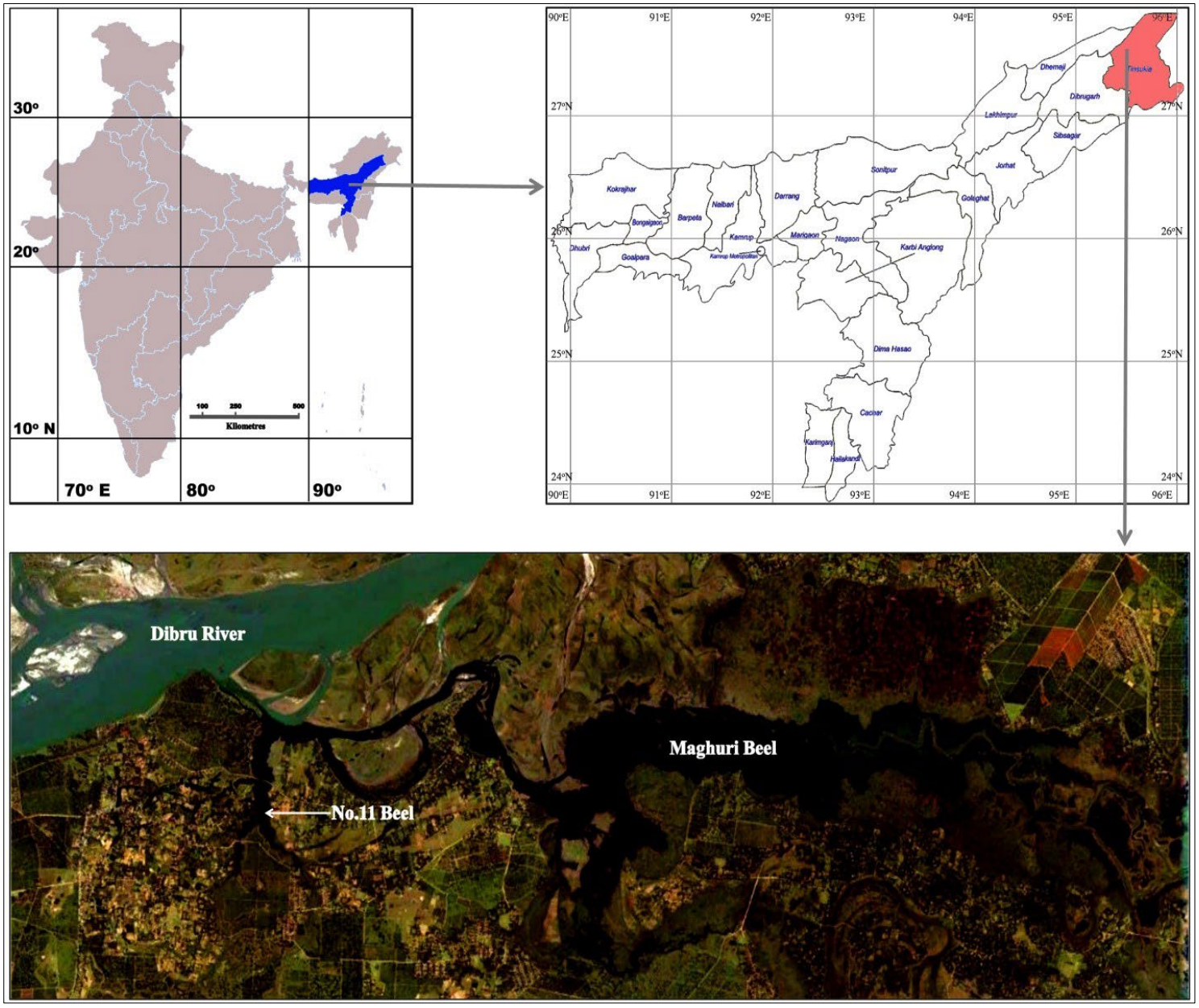


Figure 1. Map of India showing Assam state indicating location of Tinsukia district and satellite map showing the sampled beels

Table 1. Abiotic parameters (Mean ± SD) of the samples

Beel→	MAGHURI BEEL		NO.11 BEEL	
Parameters↓	**Range**	**Mean ±SD**	**Range**	**Mean ±SD**
Rainfall (mm)	0.0 - 615.0	188.4 ±193.6	0.0 - 615.0	188.4 ±193.6
Water temperature (°C)	15.0 -30.8	24.7 ±4.6	15.5 - 30.7	25.4 ±4.6
pH	6.51 - 8.26	7.38 ±0.50	6.39 - 8.72	7.42 ±0.54
Specific conductivity (µS/cm)	69.0 - 140.0	100.0 ±19.4	46.0 - 139.0	84.7 ±22.3
Dissolved Oxygen (mg/L)	4.0 - 8.0	6.0 ±1.4	4.0 - 8.0	5.6 ±1.2
Free Carbon-dioxide (mg/L)	10.0 - 28.0	15.8 ±5.0	10.0 - 24.0	16.1 ±3.8
Total alkalinity m(g/l)	40.0 - 80.0	58.9 ±12.9	38.0 - 80.0	52.4 ±10.0
Total hardness (mg/L)	54.0 - 96.0	72.6 ±10.5	50.0 - 100.0	69.2 ±10.7
Calcium hardness(mg/L)	14.7 - 25.2	20.1 ±2.8	12.6 - 25.2	18.8 ±3.7
Magnesium hardness(mg/L)	7.00 - 17.71	12.75 ±2.60	8.07 - 18.69	12.24 ±2.44
Chloride hardness (mg/L)	7.99 -20.97	13.23 ±3.43	10.98 - 24.98	16.52 ±3.67
DOM (mg/L)	0.041 -0.131	0.101 ±0.027	0.045 - 0.131	0.097 ±0.022
TDS (mg/L)	0.080 -0.320	0.160 ±0.075	0.040 - 0.320	0.155 ±0.077
Phosphate (mg/L)	0.134 - 0.322	0.189 ±0.054	0.136 - 0.371	0.194 ±0.062
Nitrate (mg/L)	0.352 - 1.881	0.733 ±0.352	0.369 - 1.550	0.720 ±0.293
Sulphate (mg/L)	6.143 - 25.047	11.020 ±5.584	5.767 - 22.907	11.482 ±5.213
Silica (mg/L)	0.657 - 1.089	0.877 ±0.188	0.661 - 1.167	0.900 ±0.192

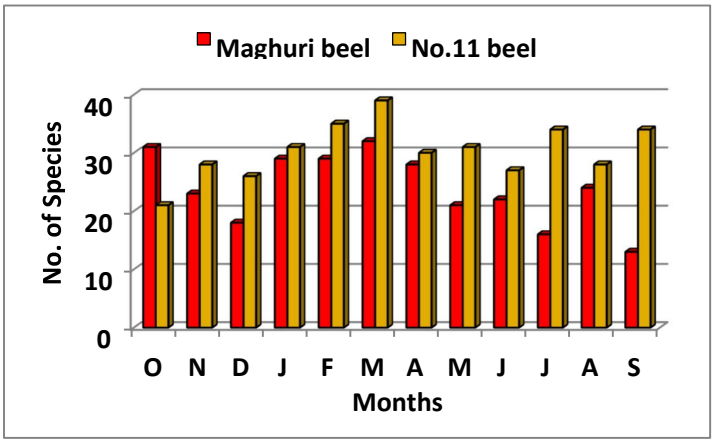


Figure 2. Monthly variation of richness of phytoplankton of DSBR beels (2013-2014)

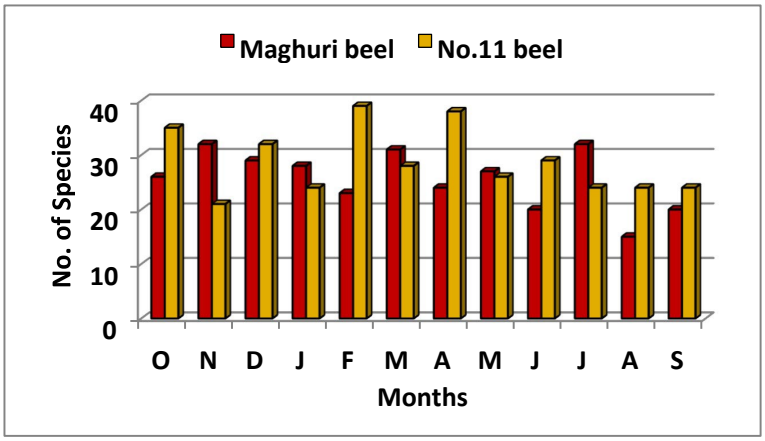
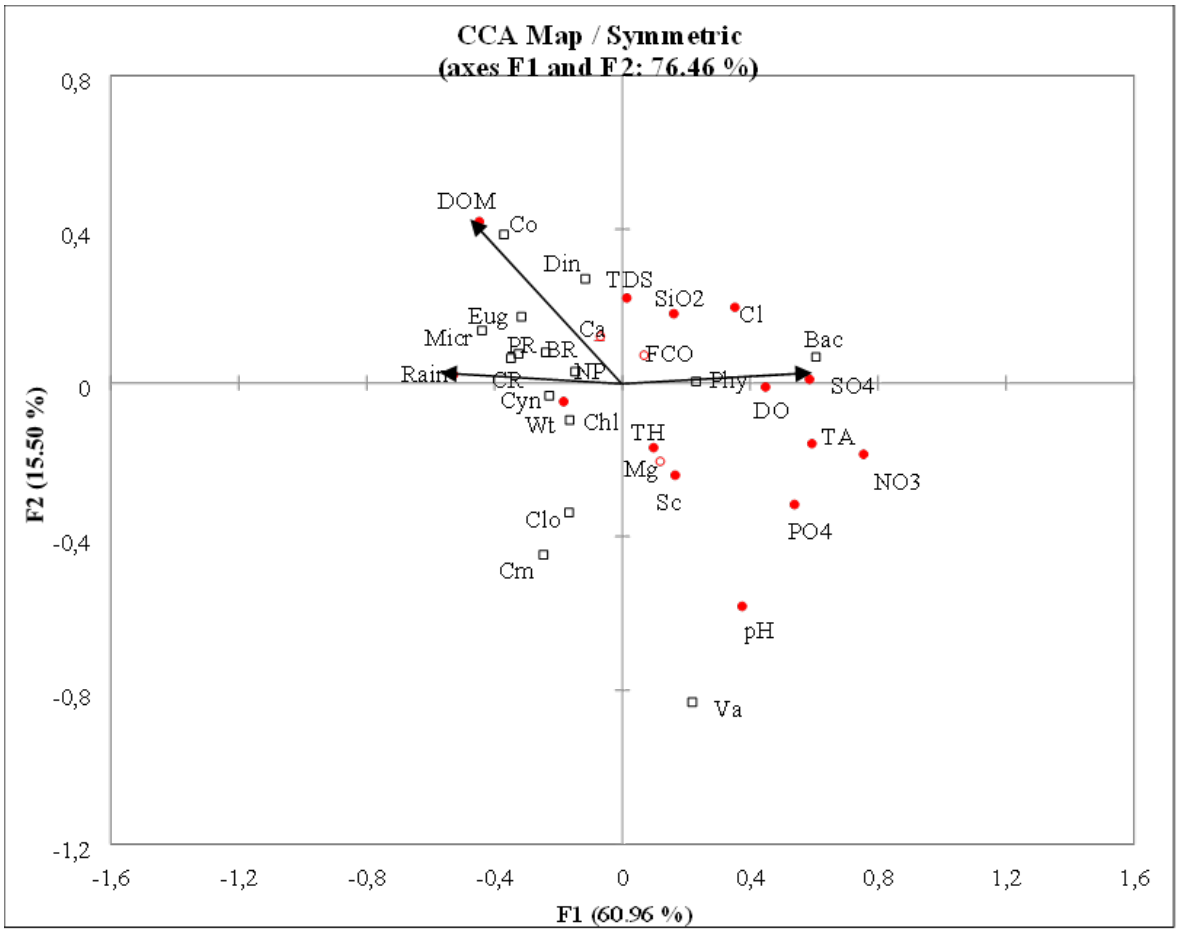


Figure 3. Monthly variation of richness of phytoplankton of DSBR beels (2014-2015)

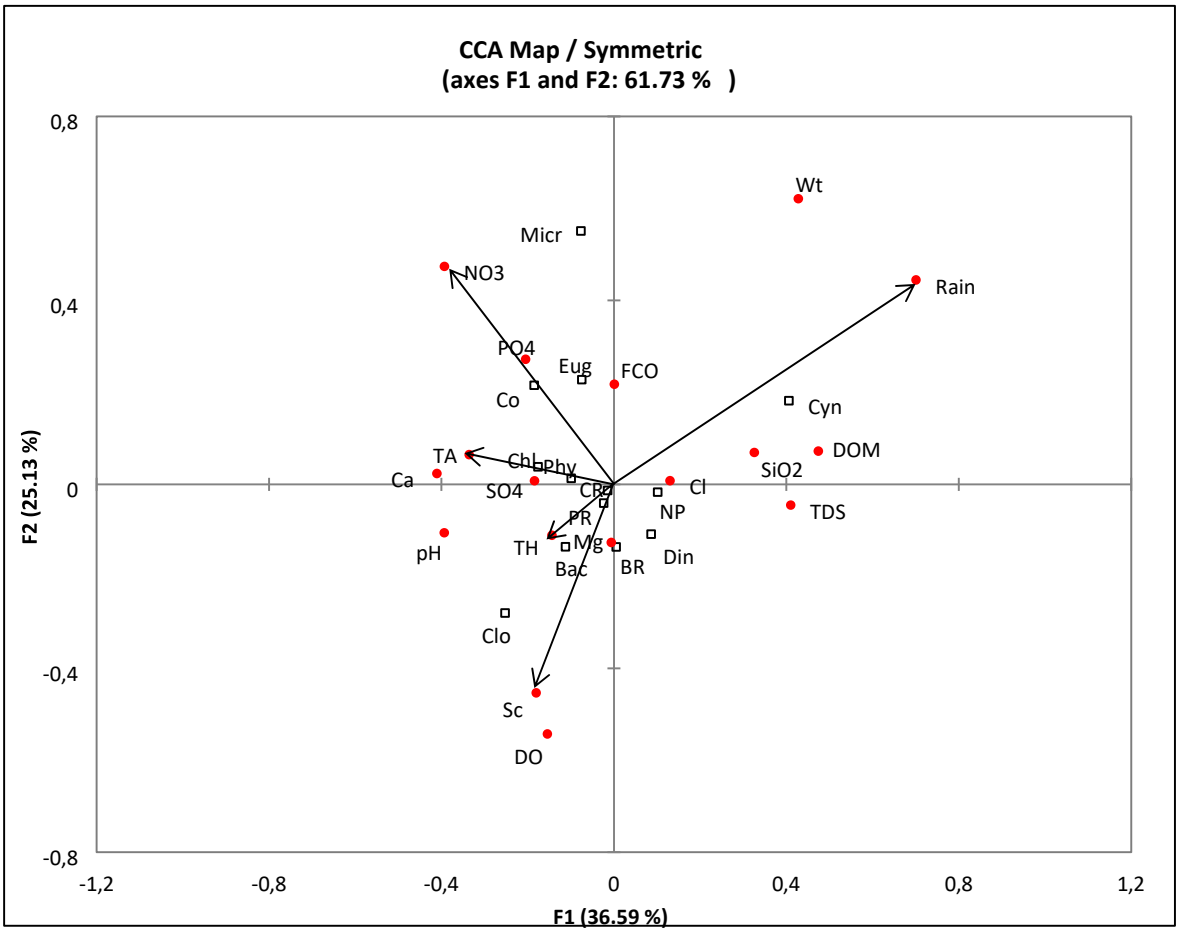
Table 2. Temporal variation of Phytoplankton between sites (October 2013 - September 2015)

QUALITATIVE	Maghuri beel		No.11 beel	
	Study period		Study period	
Net plankton total	**241 species**		**251 species**	
Net plankton	58-122	81±14	65-139	94±20
Phytoplankton Total	61species		61species	
% similarity	14.6-77.2		36-74.7	
Phytoplankton	13-32	25±6	21-39	30 ±5
Chlorophyta	5-17	12 ±4	10-24	15 ±3
Bacillariophyta	4-12	7 ±2	4-10	7 ±2
Cyanophyta	0-4	2 ±1	1-5	3 ±1
Dinophyta	0-1	1 ±1	0-1	1 ±0
Euglenophyta	1-5	3 ±1	1-7	3 ±2
QUANTITATIVE				
Net plankton n/L	214-950	359 ±150	230-438	337 ±52
Phytoplankton	39-811	162 ±157	81-243	138 ±39
% composition	17.0-85.4	39.7 ±15.8	26.7-66.7	41.0 ±9.9
Diversity	1.161-3.012	2.570 ±0.446	2.812-3.401	3.133 ±0.165
Dominance	0.104-0.776	0.255 ±0.161	0.069-0.180	0.116 ±0.031
Evenness	0.335-0.950	0.81 3±0.142	0.884-0.962	0.930 ±0.018
Different Groups				
Chlorophyta	21-128	61 ±32	39-129	66 ±23
% composition	15.4-70.9	45.6 ±15.6	36.0-63.3	47.8 ±7.6
Bacillariophyta	7-652	85 ±132	18-81	45 ±16
% composition	16.7-80.4	41.3 ±20.1	18.0-44.8	32.2 ±6.9
Cyanophyta	0-30	10 ±8	2-29	10 ±8
% composition	0.0-20.0	7.6 ±5.7	1.8-24.8	7.5 ±5.7
Dinophyta	0-3	1 ±1	0-4	1 ±1
% composition	0.0-4.2	0.7 ±1.0	0.0-2.8	0.8 ±0.9
Euglenophyta	1-11	5 ±3	5-44	16 ±9
% composition	0.8-13.1	4.7 ±3.6	4.0-26.3	11.6 ±5.7
Important taxa (n/L)				
***Cosmarium* spp.**	0-36	9 ±9	0-30	11 ±7
***Closterium* spp.**	0-44	15 ±11	3-31	13 ±7
***Micrasterias* spp.**	0-14	5 ±4	0-30	1 1±9



Abbreviations: **Abiotic:** Ca (Calcium), Cl (Chloride), DOM (dissolved organic matter), DO (dissolved oxygen), FCO2 (free carbon dioxide), Rain (rainfall), NO_3 (nitrate), PO_4 (phosphate), SiO_2 (silicate), Sc (specific conductivity), SO_4 (sulphate), TA (total alkalinity), TDS (total dissolved solids), TH (total hardness), pH (hydrogenion concentration), Wt (water temperature). **Biotic:** Bac (Bacillariophyta), BR (Bacillariophyta richness), Chl (Chlorophyta), CR (Chlorophyta richness), Clo (*Closterium*), Cm (*Closterium moniliferum*), Co (*Cosmarium*), Cyn (Cyanophyta), Din (Dinophyta), Eug (Euglenophyta), Micr (*Micrasterias*), NP (Net Plankton), Phy (Phytoplankton), PR (Phytoplankton richness), Va (*Volvox aureus*).

Figure 4. CCA coordination biplot of phytoplankton and abiotic factors of Maghuri beel



Abbreviations: **Abiotic:** Ca (Calcium), Cl (Chloride), DOM (dissolved organic matter), DO (dissolved oxygen), FCO_2 (free carbon dioxide), Rain (rainfall), NO_3 (nitrate), PO_4 (phosphate), SiO_2 (silicate), Sc (specific conductivity), SO_4 (sulphate), TA (total alkalinity), TDS (total dissolved solids), TH (total hardness), pH (hydrogenion concentration), Wt (water temperature). **Biotic:** Bac (Bacillariophyta), BR (Bacillariophyta richness), Chl (Chlorophyta), CR (Chlorophyta richness), Clo (*Closterium*), Co (*Cosmarium*), Cyn (Cyanophyta), Din (Dinophyta), Eug (Euglenophyta), Micr (*Micrasterias*), NP (Net Plankton), Phy (Phytoplankton), PR (Phytoplankton richness).

Figure 5. CCA coordination biplot of Phytoplankton and abiotic factors of No. 11 beel

Conclusion

To sum up, phytoplankton communities of DSBR beels are diverse and speciose and are characterised by Chlorophyta's qualitative and quantitative importance. Bacillariophyta, the second most diverse group after Chlorophyta, also contributed significantly to phytoplankton abundance in the sampled beels. The species diversity of phytoplankton is influenced by richness and equitability or relative abundance of species. Phytoplankton communities depicted higher species diversity, evenness and lower dominance. The present study recorded minimal influence of individual abiotic factors. CCA results suggested the cumulative importance of seventeen abiotic factors vis-à-vis variations of phytoplankton assemblages of the floodplain lakes of DSBR.

Compliance with Ethical Standards

Conflict of interest: The author(s) declares that they have no actual, potential, or perceived conflict of interest for this article.

Ethics committee approval: -

Data availability: Data will be made available on request.

Funding disclosure: -

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Disclosure: -

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AQUATIC RESEARCH

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Aquat Res 6(4), 271-275 (2023) • https://doi.org/10.3153/AR23026 *Short Communication*

The first report and a new host record of leech fish, *Trachelobdella lubrica* (Grube, 1840) infecting the gills of *Sparus aurata* (Linnaeus, 1758) from the Gulf of Bejaia, Algeria

Souhila RAMDANI

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University Abderrahmane Mira Bejaia, Faculty Life Sciences, Algeria

ORCID IDs of the author(s):
S.R. 0000-0003-4436-2620

ABSTRACT

This study is to be the first report and new host record of segmented worms in the family Piscicolidae, *Trachelobdella lubrica* parasitizing *Sparus aurata* off the coasts of Algeria. *Sparus aurata* constitutes new host record for *Trachelobdella lubrica.* 05 specimens of *Sparus aurata* were examined for their leech parasites. A single specimen of leech species was recovered from the gills of *Sparus aurata*. Typical characters allowed us to classify the leech as *Trachelobdella lubrica.*

Keywords: First report, *Trachelobdella lubrica*, New host, *Sparus aurata*, Algeria

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Correspondence:
Souhila RAMDANI
E-mail: Souhilaramdani.dz@gmail.com

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Introduction

Leech in fish is well-known as ectoparasites that can attach directly to the main body of the fish (Bielecki et al., 2008) and to various sites on the body of the host, including the pectoral, pelvic, dorsal, and caudal fins (Bielecki et al., 2011; Kaygorodova et al., 2011; Schulz et al., 2011), the gill cavities (Volonterio et al., 2004; Oktener and Utevsky, 2010), the eyes (Murwantoko et al., 2018) and the mouth cavity (Cruz-Laccierda et al., 2000).

Leech infections can cause mortality from physical trauma and blood loss, predisposing hosts to secondary infections and transmitting pathogenic viruses, bacteria and flagellated haemoprotistans (Negm Eldin, 1995; Opara, 2002). *Piscicola geometra* was shown to transmit SVC virus to Carp (Ahne, 1985). Feeding wounds may become contaminated by opportunistic bacteria and fungi (Kabata, 1985).

Fish leeches of the Mediterranean Sea have been explored mainly from the Türkiye Sea (Oktener and Utevsky, 2010; Yanar et al., 2019), from Italy water (Bottari et al., 2017; Liuzzo et al., 2018) and Tunisia (Ben Ahmed et al., 2015).

In Algerian water, leeches in fish have never been recorded. A single specimen of marine leech species was recovered from the gills of *Sparus aurata* (Linnaeus, 1758) from the Gulf of Bejaia. Typical characteristics of this leech species allowed us to identify the leech as *Trachelobdella lubrica* (Grube, 1840).

To our knowledge, the presently reported *S. aurata* constitutes a new host record for the marine leech, and this is the first report of this parasite from the Mediterranean waters off the Algerian coast. This paper deals with a preliminary analysis of the morphology of the parasite leech.

Material and Methods

Fish samples, ranging between 375 to 525g, were obtained in the harbour from commercial boats fishing in the Gulf of Bejaia from February to May 2022. Fish specimens (n=05) were transported to the laboratory (University of Bejaia) in the more incredible container. Infected fish was photographed, and the parasite (P=20%) was removed from the host and fixed in ethanol for further morphological examinations, according to Epshteĭn, 1973 Sawyer et al. 1975 and Burreson, 2020.

Results and Discussion

This paper is the first documented report of marine fish leech infestation on the Algerian coast, with *Sparus aurata* as a new host record for the leech parasite *Trachellobdella lubrica*. The marine leech *T. lubrica* has been observed to infest several marine fishes in the Mediterranean Sea, from Tunisia (Ben Ahmed et al., 2015), Türkiye Sea (Saglam et al., 2003; Oktener and Utevsky, 2010), from the Italian coast Italy (Ghion et al., 1982) and from Israel water (Gabel et al., 2020). *T. lubrica* is abundant in all biogeographic regions, along the coast of Australia, the Philippines, the Hawaiian Islands, the United States, the Gulf of Mexico, Puerto Rico, the Canary Islands and New Zealand (Epshtein, 1973; Sawyer et al., 1975; Ernest et al., 1994; Garcés, 1995; Burreson et al., 2006; Burreson, 2020).

T. lubrica is a common parasite attaching to the gill cavity in numerous marine species of teleost fishes. The host *S. aurata* constitutes a new host record for *T. lubrica*. The host list of *T. lubrica* is presented in Table 1.

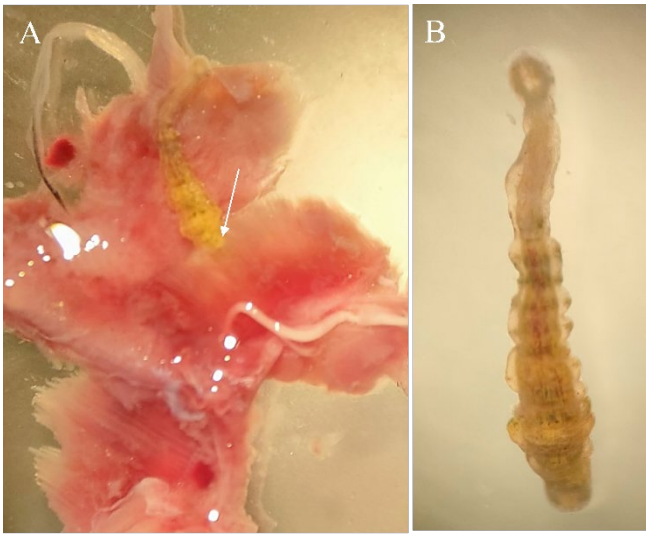

Figure 1. A: leech on gill aches of *Sparus aurata* (white Arrow*)*
B: leech total view. Scale bar: A, B, =1cm

Conclusion

The parasitic leech fauna in fish still needs to be studied in Algeria; further studies are necessary on parasitic leech fish in Algeria.

Table 1. List host species of leech parasite *Trachelobdella lubrica*

Leech specie	Host species	Locality	References
Trachelobdella lubrica	*Fistularia petimba*	Australia	Epshtein, 1973.
	Fistularia villosa	Philippine	
	Epinephelis guernus	Hawaiian Islands	
	Priacanthus boops		
	Priacanthus melki		
	Priacanthus cruentatus		
	Caranx adsensionis		
	Trachurus trachurus strachurus		
	Trachurus trachurus capensis		
	Sciaena umbra		
	Umbrina cirrosa		
	Lethrinus miniatus		
	Lethrinus nebulosus		
	Acanthopagrus bifasciatus		
	Diplodus annularis		
	Upeneus sulphureus		
	Paristiopterus gallipavo		
	Labrus sp.		
	Coris julis		
	Uranoscopus scaber		
	Nemadactylus macropterus		
	Blennius sanguinolentus		
	Siganus oramin		
	Gobius niger		
	Scorpaena scrofa		
	Scorpaena porcus		
	Chelidonichthys kumu		
	Taurulus bubalis		
	Solea solea		
	Lophius piscatorius		
	Lutjanus cyanopterus	United States Gulf of Mexico	Sawyer et al., 1975.
	Dicentrarchus labrax	Italy	Ghion et al., 1982.
	Pomacentrus partitus	Puerto Rico	Ernest et al., 1994.
	Sciaenops ocellatus	/	Garcés, 1995.
	Scorpaena porcus	Türkiye	Saglam et al., 2003.
	Scorpaena scrofa /	Canary Islands	Burreson et al., 2006.
	Labrus bergylta,	Türkiye	Oktener and Utevsky, 2010.
	Diplodus vulgaris		
	Epinephelus aeneus		
	Symphodus tinca	Tunisia	Ben Ahmed et al., 2015.
	Lethrinus Laticaudis	Australia	Burreson, 2020.
	Lutjanus sebae	New Zealand	
	Epinephalus merra		
	Lethrinus nebulosus		
	Scorpaena cardinalis		
	Lagocephalus sceleratus	Israel	Gabel et al., 2022.
	Sparus aurata	**Algeria**	**Present study**

Compliance with Ethical Standards

Conflict of interest: The authors declare that for this article, they have no actual, potential, or perceived conflict of interest.

Ethics committee approval: Ethics committee approval is not required.

Data availability: Data will be made available on request.

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Disclosure: -

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