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Analysis of Plankton Intake to the Growth and Omega-3 Fatty Acid Content of Milkfish (*Chanos chanos* Forsskal, 1775)

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ABSTRACT

The aim of the present study was to examine the food, growth, and omega 3 fatty acid content of milkfish (*Chanos chanos*) in Sidoarjo fish pond, East Java. The study was conducted by spreading 5,000 fish milkfish seeds (5-7 cm long and 0.6-1.0 g) to the fish pond. The length and weight of milkfish were measured, and the digestive tract was isolated. For sampling, a plankton net was used and samples were preserved in 1% Lugol solution. Data were analyzed to determine the food type and selection, growth rate, relative intestine length, and weight length relationship. The recorded relative intestine length of milkfish was 5.4-6.4 cm and was categorized as herbivorous fish. The food type was phytoplankton with a length of <14 cm and zooplankton with a length of >15 cm. The proportion of zooplankton in the fish intestine increased with its size. The phytoplanktons found dominantly in the digestive tract were *Chaetoceros* sp. and *Nitzschia* sp. and zooplankton were *Calanus* sp. and *Cyclop* sp. The daily length growth rate ranged from 0.008 to 0.283 cm, while the daily weight growth rate ranged from 0.5 to 1.16 g. The length-weight relationship of milkfish (*Chanos chanos*) can be determined by equation W=0.1466x with the regression index R2=1. Thus, milkfish (*Chanos chanos*) tended to have isometric growth patterns with the length and weight and were directly proportional to the omega-3 content in the milkfish body.

INTRODUCTION

Milkfish (*Chanos chanos)* is a beneficial source of fatty acid required for health. **Agustini *et al*. (2010),** mentioned that *Chanos chanos* is a source of unsaturated fat in the form of omega-3 with a percentage of 19.56, 7.4% omega-6, and 19,24% omega-9. The high content of omega-3 in this fish is due to excistance of plankton and microalgae that produce a lot of omega-3 and fatty acids and are eaten by the specific fish **(Estiasih, 2009)**. Fish has significant chemical content including high protein. Futhermore, milkfish fat has more double bond, which is an unsaturated fatty acid. Omega-3 are an unsaturated fatty acids that do not convert to cholesterol in the body, Tus it can decrease blood cholesterol levels **(Suptijah, 1999)**.

Recently, diet with enough sea fish, especially phytoplankton-eating fish, can reduce the risk of coronary heart disease because it inhibits the atheroclerosis process by reducing blood cholesterol, triglyceride and LDL levels and also increasing HDL. Futhermore, the potentials of omega 3 fatty acids to affect the immune system **(Calder, 2007; Chapkin *et al*. 2009)** by improving the cell membrane function and increasing low density lipoprotein (LDL) oxidation **(Mahrus, 2008)**. It can also suppress or reduce the risk of chronic hereditary diseases has been proven, including diabetes **(Rudkowska, 2010),** coronary heart disease **(Car & Webel, 2012; Xin *et al*, 2012),** atherosclerosis **(Miles & Calder, 2012),** anti-cancer, tumors **(Cockbain *et al*. 2012; Vaughn *et al*. 2013)** and decrease blood cholesterol.

The composition of fats and fatty acids in fish varies in accordance to species, season, geographical location, gonad maturity level and fish size **(Stansby, 1967; Kusumo, 1997)**. Markedly, marine phytoplankton is a primary source of omega 3 in the food chain. PUFAs derived from marine organisms usually exists in the form of triglycerides, although it can be in other forms such as esters or phospholipids **(Berge & Barnathan, 2005)**. Fish oil from the Scombroidae, Clupeidae and Salmonidae families has the highest EPA and DHA **(Rodriguez *et al*. 2010)**. Milkfish (*Chanos chanos*) is a common type of plankton-eating fish with the main food of diatoms, filamentous green algae and detritus **(Rao & Sivani 1996; Franklin *et al*. 2006; Prayitno *et al*. 2015)**. The existence of *Chanos chanos* is act mainly to control plankton populations and increase the catch of fishermen at Ir. H. Djuanda Reservoir in July to August 2008 was about 2,116,000 fishes **(Tjahjo & Purnamaningtyas, 2009)**. The introduced *Chanos chanos* in those waters are able to utilize plankton abundance and possess a very fast growth rate ($K = 3.381$; with $L\infty = 45$ cm).

Chanos chanos has a very wide salinity tolerance, ranging from saltwater (35 ppt) to freshwater (0 ppt), so that it can be maintained in saltwater until freshwater **(Coad, 2015)**. *Chanos chanos* as a commodity of brackish and saltwater has been widely known in the community for a long time **(Chong *et al.*, 1984)**. This fish has been used as a source of animal protein for coastal communities and trade commodities with a quite high price. *Chanos chanos* is a very important source of animal protein in Southeast Asia too, and is widely cultivated in fishpond areas in Philippines, Indonesia and Taiwan. The length of the fish in sea waters is ± 100 cm and can reach to a maximum of 180 cm **(Nelson *et al.* 2016)**. Thus, the purpose of this study was to examine the food, growth and omega- 3 fatty acids content from milkfish (*Chanos chanos*) in fish pond at Sedati District, Sidoarjo, East Java.

MATERIALS AND METHODS

1. Milkfish seeds

Milkfish seeds were obtained from Marine and Fisheries Polytechnic and fish ponds at Sedati District, Sidoarjo (Fig.1) with average length of 5-7 cm, weight of 0.6-1.0 g and age of about 3 months. The seeds harvested are temporarily accommodated in an aerator equipped reservoir. Transported with a closed system using plastic bags that were given oxygen. In each plastic bag, a volume of 30 litres was added to ± 500 *Chanos chanos* seeds, then pure oxygen gas was added to the water: air with a ratio of 1: 2.

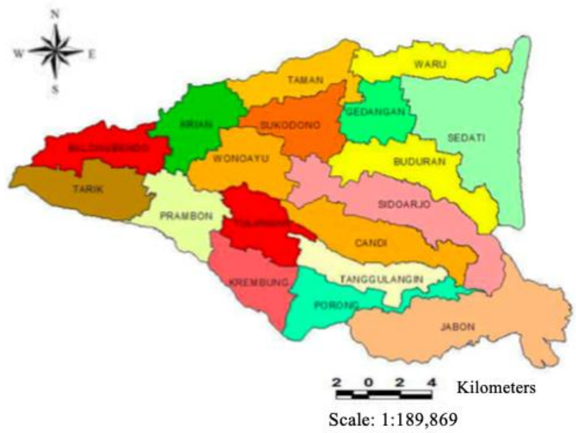

Fig. 1. Sidoarjo Map **(Faradina *et al.* 2015)**

Chanos chanos specimens were obtained by catching once a month from March to August 2018, using 1.25; 1.5; 1.75; 2.0 inches mesh size of gill net with the help of local fishermen. Each specimen was stored in a fish storage box and taken to a laboratory to measure the total length and weight, and analyze the contents of the digestive system. The total length was measured from the tip of the upper jaw to the tip of the tail using a metal bar to the nearest millimetre. Weight of fish was weighed using an electrical scale with a precision of 0.1 gram. The type of *Chanos chanos* was observed by opening the stomach, removing intestinal contents, then identifying the species under a microscope using a plankton identification manual **(Shirota 1996; Verlencar 2004; Barsanti & Gualtieri, 2006).**

2. Sampling of Plankton

Plankton sampling was carried out by filtering water using an 86 μm eye opening plankton net. Water was taken using a 5 litre- bucket six times so that the total filtered water was 30 lites, then 30 ml filtered water was added lugol to 4% content and stored in plastic bottles. Plankton sampling location was the same as the location of the installation of gill nets.

3. Plankton abundance

Each bottle of preserved plankton sample was shaken by flipping the bottle and then 1 ml was taken with the use of a pipette, then carefully poured evenly into the Sedgwick-Rafter Counting Cell. Plankton samples were examined under light microscope. Using taxonomic keys, the species or the genera level was identified and calculated **(Shirota 1996; Verlencar 2004; Barsanti & Gualtieri, 2006)**. The total abundance of plankton was calculated using equation (1): Formula of total abundance of plankton was calculated as follows:

$$N = ni \times (Vr/V_0) \times (1/V_s) \times 1000 \qquad (1)$$

Note: N = total number of individuals (cells, ind) plankton/liters, ni = Number of cells or individuals observed in the n= 1 species, V_r = volu`me of filtered water (30 ml), V_o = volume of water observed at Sedgwick- Rafter (1 ml), V_s = volume of filtered water (30 liters), 1000 = conversion of litres into m^3 units.

4. Food

The relative proportion of the intestinal length to body length is used to determine the trophic level of fish. Based on the relative intestinal length, fish are divided into carnivores (<1), omnivores (1-3), and herbivores (> 3) **(Biswas, 1993)**.

$$\text{Relative intestinal length} = \frac{\text{intestinal length}}{\text{body lenght}} \qquad (2)$$

The largest index was used to analyze the types of fish food and assess the types of food most eaten by fish. The largest part index analysis was calculated according to equation (3):

$$I_i = \frac{Vi.Oi}{\sum_{n}^{i=1} Vi.Oi} \; x\ 100 \qquad (3)$$

Note: I_i = the largest index, V_i = percentage of volume of i-type fish food, O_i = percentage of frequency occurrence of n-type food, n = number of fish food organisms (i = 1,2,3, ... n).

5. Growth in Length and Weight

The average length of *Chanos chanos* was calculated using equation (4):

$$L_t = \sum \frac{Li}{n} \qquad (4)$$

Note: L_t = average length (cm) of fish at age t (sampling time), L_i = length of fish i, n = number of fish measured in length.

The average weight of *Chanos chanos* was calculated using equation (5):

$$W_t = \sum \frac{Wi}{n} \qquad (5)$$

Note: W_t = mean weight of fish at age t (g), W_i = weight of fish to i (g), n = number of fish weighed.

6. Omega 3 Fatty Acids Content

Fatty acid extraction was carried out following the method of **Bligh and Dyer (1959)** after methylated using themethod of **Morisson and Smith (1964)**. Three *Chanos*

chanos from each sampling were taken and the abdomen were cleaned. Subsequently, they were left in the sun to dry. The dried fish were taken in the Dorso-lateral part of the body and mixed, then weighed as much as one gram, then crushed in a glass cup and extracted by adding 10ml of petroleum ether (PE), centrifuged at 3000rpm for 2 minutes and filtered. Next, the mixture was allowed to stand for about 15 minutes so that the petroleum ether would evaporate and fat was obtained. The fat was then methylated by adding 10ml of BF3 in methanol (BF3CH3OH) 20% then was shaken with a shaker for about 30 minutes. The top layer, containing the fatty acid methyl ester, was separated and analyzed using Gas Chromatography (GC).

RESULTS

1. **Milkfish *Chanos chanos* seeds**

The number of the stocking fish was 5,000 specimens . The *Chanos chanos* had a total length range of 3.5-5.0 cm with an average of 4.21 ± 0.45 cm and weight of 0.3-1.0 g with an average of 0.55 ± 0.20 g. *Chanos chanos* were first caught using gill nets with 1.25-inch eye openings net in mid-March. They were grouped into four classes with a diameter of 5 cm, and the smallest group got lengths <15 cm, 16-20 cm, 21-25 cm, and the largest were > 26 cm.

2. **Types of Plankton**

The composition of plankton species in fish ponds at Sedati district, Sidoarjo is presented in Table (1). Nine species of phytoplankton were found in ponds; namely, *Chaetoceros* sp., *Nitzschia* sp., *Spirogyra* sp., *Surirella elegana, Synedra, Tabellaria* sp., *Melosira* sp., *Navicula* sp. and *Pleurosigma* sp. While zooplankton in the pond were eight species, including *Branchionus* sp., *Calanus* sp., *Copepoda* sp., *Cyclop* sp., *Daphnia* sp., *Diaptomus* sp., *Euglypha* sp., and *Euterpina* sp. Phytoplankton species most found were *Chaetoceros* sp., but with respect to zooplankton, *Cyclop* sp was the most identified.

3. **The presence of types of food**

The types of phytoplankton found in the *Chanos chanos* digestive tract consisted of *Chaetoceros* sp., *Nitzschia* sp., *Pleurosigma* sp., *Spirogyra* sp., *Surirella* and *Synedra* sp. The zooplankton species found were *Branchionus* sp., *Calanus* sp., *Cyclop* sp., *Daphnia* sp., and *Diaptomus* sp. The presence of food types from the phytoplankton group ranged from 31.31% to 67.24% ,and the most abundant was *Chaetoceros* sp. In the zooplankton group, the presence of zooplankton foods ranged from 46.55 to 69.83%. The variation in the presence of types of food in the phytoplactone group was greater than in the zooplankton group.

4. **Food Types Composition**

The composition of the types of food consumed by *Chanos chanos* consisted of phytoplankton and zooplankton (Table 2). It appears that the larger the size of the fish, the more type of food is turned into zooplankton.

Table 1. The mean abundance of phytoplankton (cell / m^3) and zooplankton (ind / m^3).

Species	April	May	June	July	August	September	Average	%
PHYTOPLANKTON								
Chaetoceros sp.	89.091	145.750	19.800	190.575	145.750	19.800	101.794	20,6
Melosira sp.	56.818	80.300	18.150	154.963	11.550	18.150	56.655	11,4
Navicula sp.	18.182	29.700	7.150	55.825	29.700	7.150	24.618	5,0
Nitzschia sp.	46.364	70.950	11.550	107.800	70.950	143.413	75.171	15,2
Pleurosigma sp.	0	0	5.000	60.638	68.750	5.000	23.231	4,7
Spirogyra sp.	50.455	74.250	17.050	143.413	74.250	17.050	62.745	12,7
Surirella sp	52.727	68.750	15.950	142.450	68.750	11.550	60.030	12,1
Synedra sp.	63.636	81.950	18.150	165.550	81.950	18.150	71.564	14,5
Tabellaria sp.	0	0	4.091	49.088	56.818	4.091	19.015	3,8
Total	**377.273**	**551.650**	**116.891**	**1.070.302**	**608.468**	**244.354**	**494.823**	
ZOOPLANKTON								
Branchionus sp.	0	0	30.250	43.313	30.250	43.313	24.521	3,5
Calanus sp.	68.182	99.550	117.700	223.300	117.700	223.300	141.622	20,2
Copepoda	0	0	27.500	55.825	69.545	55.825	34.783	5,0
Cyclop sp.	79.545	123.750	145.750	263.725	145.750	79.545	139.678	20,0
Daphnia sp.	75.000	107.250	77.000	140.525	77.000	140.525	102.883	14,7
Diaptomus sp.	69.545	88.000	115.500	212.713	79.545	212.713	129.669	18,5
Euglypha sp.	65.909	83.600	86.900	153.038	86.900	153.038	104.898	15,0
Euterpina sp.	0	0	14.300	30.800	14.300	69.545	21.491	3,1
Total	**358.181**	**502.150**	**614.900**	**1.123.239**	**620.990**	**977.804**	**699.544**	

Table 2. The composition of *Chanos chanos* food based on length of fish groups.

Length class (cm)	Number of sample	Mean weight (g)	Dominant food group (%)	Dominant food type (%)
<15	9	17,4	Fitoplankton (96)	*Nitzschia* sp. (62)
16-20	6	50,2	Fitoplankton (84)	*Chaetoceros* sp. (83)
21-25	30	95,6	Zooplanton (68)	*Cyclop* sp. (37)
>26	19	150	Zooplankton (58)	*Calanus* sp. (38)

5. Food composition and selection

The composition of plankton species in pond waters and *Chanos chanos* food types is presented in Fig. (2). There were 9 species of phytoplankton in pond waters, while the phytoplankton species found in *Chanos chanos* intestines were 6 species. Similarly, zooplankton that exist in waters were 8 species, while those found in the intestines of *Chanos chanos* were only 5 species. Phytoplankton that were not found in the intestines of *Chanos chanos* were: *Melosira* sp., *Navicula* sp., and *Tabellaria* sp., while zooplankton that were not found in the intestines of *Chanos chanos* were *Copepoda* sp., *Euglypha* sp., and *Euterpina* sp.

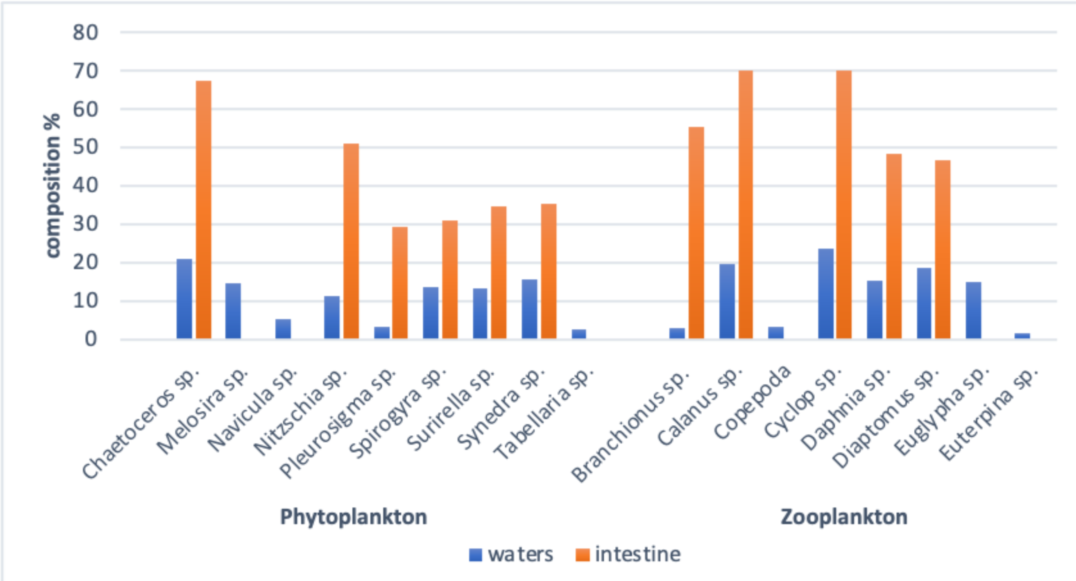

Fig. 2. Proportion of types of plankton in water ponds and intestines of *Chanos chanos*.

6. Relative length intestines

Relative intestine length indicates the type of food consumed by *Chanos chanos* in ponds which is presented in Table (3). Based on the average relative intestinal length, *Chanos chanos* is classified as herbivorous fish. The greater the length of *Chanos chanos*, the longer the average length of the intestine is.

Table 3. The relative length of *Chanos chanos* intestine based on the length groups in Sedati District of Sidoarjo.

Length class (cm)	Number of sample	Intestine length (cm)	Average intestine length (cm)	Relative intesine length	Average relative intestine length	Note
<15	7	59-83	70.4	4.4-5.7	5.4	Herbivore
16-20	22	81-134	104.5	4.3-6.8	5.5	Herbivore
21-25	70	94-86	137.7	4.1-8.7	5.8	Herbivore
>26	17	152-200	174	5.7-6.9	6.4	Herbivore

7. Growth Length and Weight of Fish

The total number of *Chanos chanos* caught during the study was 125 fishes. The number of samples captured each sampling month from April to September were 8, 15, 24, 26, 30, 22, respectively, with a range of 3.5-29.5 cm long and weights of 0.9-197.5 g.

The growth and length of the absolute weight of *Chanos chanos* every month are presented in Fig. (3). In general, the length and weight of *Chanos chanos* measured every month gave a rapid change in length and weight from the time of stocking (April) till November. At the beginning of the dry season (July), fish expressed a slowing down in length and weight growth.

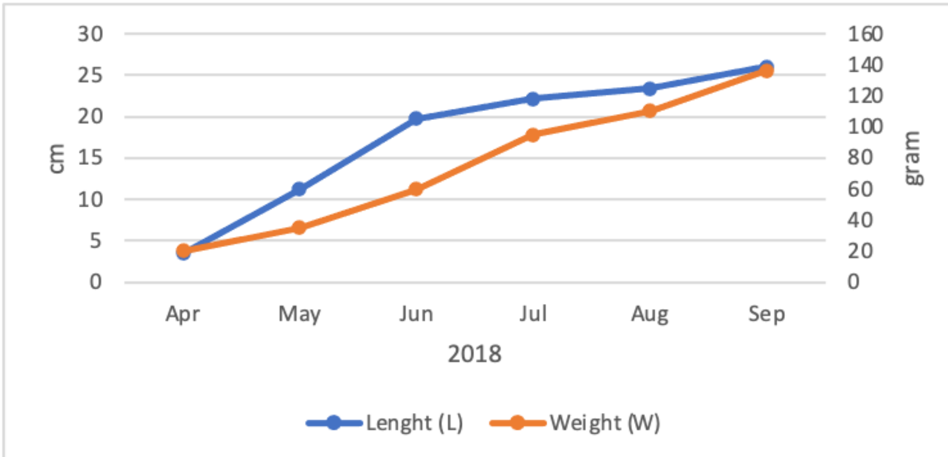

Fig. 3. Mean length and weight of *Chanos chanos* in April-September 2018.

8. The Relationship of Length and Omega-3 fatty acid content

The number of *Chanos chanos* samples used for the analysis of the lenght-weight relationship were 125 fishes, which is the total catch during sampling in March – August. The lenght-weight relationship of *Chanos chanos* is presented in Fig. (4).

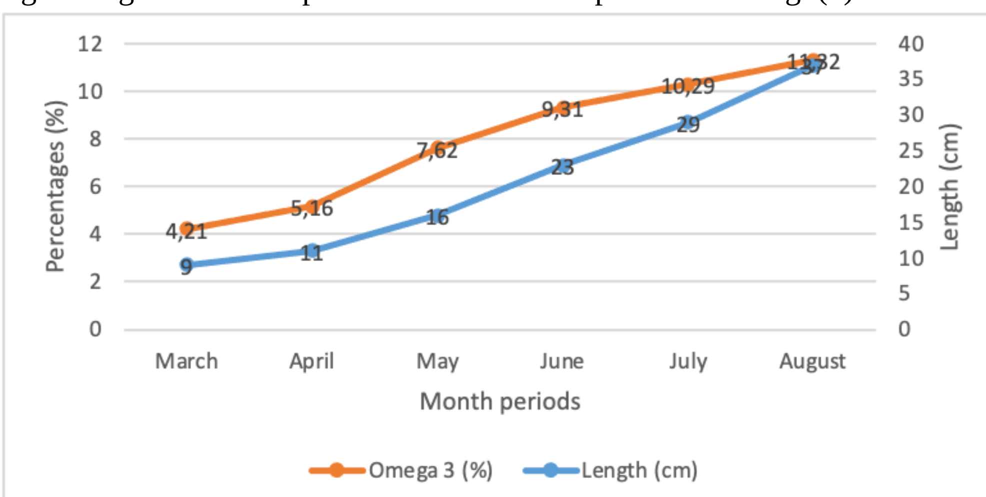

Fig. 4. Omega-3 fatty acid content and length of *Chanos chanos.*

The analysis of omega-3 fatty acids from the *Chanos chanos* body recoded a relatively high omega-3, that were seen from various lengths of fish (Fig. 3). It is worth mentioning that, the length and weight of the *Chanos chanos* are directly proportional to the omega-3 content in the body. The longer the body of the fish, the greater the omega-3 is in the body. In this context, the biggest *Chanos chanos* caught in September with an average size of 26 cm contained omega-3 of 11.32% of the total fatty acids in the whole body .

DISCUSSION

The number of phytoplankton found in pond waters were nine species and zooplankton were eight species, while the density of phytoplankton ranged from 117 to 1070 cells/L and zooplankton ranged from 358 to 1153 ind/L. Phytoplankton predation by *Chanos chanos* was very high as indicated by the average frequency of types of food found in the digestive tract of 125 fish of *Chanos chanos* by 41.4 ± 14.8% (Table 3), thus causing a very low phytoplankton density.

Phytoplankton growth requires adequate nutrients for metabolism. The most needed nutrients in water with regard to phytoplankton are carbon, nitrogen, and phosphorus. Each phytoplankton cell contains about 50% protein and about 7-10% nitrogen **(Barsanti & Gualtieri, 2006)**. Phytoplankton need nitrogen for cell wall formation, cell metabolism, amino acid formation. Notably, nitrate is a stable compound of nitrogen which is an important nutrient for autotrophic organisms and is known as a limiting factor for growth. Nitrogen and phosphorus levels become a limiting factor if nitrogen levels are <0.02 mg/L and phosphorus levels are <0.005 mg/L. Comparison of nitrogen and phosphorus levels if they are <12, then nitrogen becomes a limiting factor for phytoplankton growth, whereas if > 12, phosphorus becomes a limiting factor for phytoplankton growth **(Geider & MacIntyre, 2004)**. In ponds, nitrate and phosphate levels every month range from 0 - 0.867 (0.069) mg/L and 0 - 1,921 (1.42) mg/L with an N/P ratio of 10.34 **(Rustadi, 2009)**. Nitrogen and phosphate compounds in pond waters are not barriers in themselves, but the ratio of N and P concentrations is barrier for growth.

Chanos chanos is a plankton-eating fish that obtains its food by filtering water of its environment using long and tight gill filters **(Coad, 2015).** In this study, *Chanos chanos* could utilize most of the phytoplankton and zooplankton in pond waters as food and energy sources, so that the length and weight of the individual increases with time. *Chanos chanos* can consume several types of phytoplankton and zooplankton found in the waters according to their stage and age. Based on the lasrgest part index, the most widely used phytoplankton was *Chaetoceros* sp. with a range of 6.3-64.0, while the zooplankton group was *Cyclop* sp. with a range of 0-24.7.

The *Chanos chanos* diet consisted of phytoplankton, zooplankton, detritus, and plant residues in percentages of 43%, 46%, and 11%, repectively **(Aqil *et al.*, 2013)**. The digestive system of *Chanos chanos* is different from other herbivorous fish, because *Chanos chanos* has a stomach that functions food storage temporarily, to stir or mix food with gastric sap and deliver food into the intestine, so that *Chanos chanos* is able to efficiently digest plankton. The entire surface of the stomach is covered by mucous cells which contain somewhat acidic mucopoly-carida, functioning as a protective wall of the stomach from the work of hydrochloric acid **(Coad, 2015).** *Chanos chanos* in ponds prey on phytoplankton and zooplankton, although the proportion of zooplankton is small. *Chanos chanos* food varies depending on the type of food available in the waters. The types of phytoplankton that are widely consumed are *Chaetoseros* sp. and *Nitzschia* sp., While the types of zooplankton are *Calanus* and *Cyclop* sp.

Chaetoceros sp. and *Nitzschia* sp. are types of lipid-producing organisms with a high lipid content reaching around 71.5% **(Geider & MacIntyre, 2004)**, thus they supply *Chanos chanos* with the required energy. Moreover, **Coad (2015)** stated that *Chanos chanos* filter plankton, eat benthic organisms, cyanobacteria, diatoms, foraminifera, filamentous green algae, detritus, shellfish, snails, worms, and some crustaceans. Remarkably, phytoplankton cell walls are composed of cellulose, while zooplankton cell walls are composed of cell membranes. Cellulose is more difficult to digest than cell membranes, and thus, takes more time to digest phytoplankton. *Chanos chanos* with a total range of 15-25 cm length minimum have intestine length of 5.4 times the total body length (Table 6), hence that food can be digested completely. In addition, according to **Bagarinao (1994),** *Chanos chanos* have small mouths without teeth, soft and dense gill filters as food filtering devices, and a pair of muscles similar to an epibranchial raker organ. The esophagus is long and thick-walled, with 20-22 spiral folds and has many mucous cells, and a big belly, very thick-walled pylorus and mucous membranes. Stomach has glands that function in digesting food ingredients. The intestine is convoluted and very long. The entire digestive tract is circular and forms a compact mass in the abdominal cavity **(Bagarinao, 1994)**.

Along with the growth of fish, *Chanos chanos* food in ponds has shifted. In small size with total length that is<14 cm or the young aged, the main food is phytoplankton dominated by *Chaetoceros* sp. While, when the size is > 15 cm length, zooplankton becomes complementary food dominated by *Calanus sp* and *Cyclop* sp. The choice of *Chanos chanos* for certain types of plankton is determined according to their needs and availability in waters. At a young age, the energy requirements for growth are very high, thus consumption of Chaetoceros sp. and Nitzchia sp. becomes very high even reaching 1.5 times the concentration in waters. Furthermore, the type of chanos varies depending on the living stage and its habitat **(Bagarinao, 1994)**.

Ontogeny of fish food that occurs in *Chanos chanos* in ponds is caused by a shift in the availability of food types and demands to meet their nutritional needs. The main food of adult *Chanos chanos* consists of benthic and planktonic organisms including gastropods, lamellibranchia, foraminifera, filamentous algae, diatoms, copepods, nematodes and detritus **(Coad, 2015)**. The relationship of length and weight of *Chanos chanos* in pond waters has an isometric growth pattern, indicating that the additional length and weight of the *Chanos chanos* are proportional. The weight of *Chanos chanos* ranges from 2.78 to 3.46 **(Froese & Pauly, 2015),** generally influenced by food availability, behavior, and habitat. The length-weight relationships of *Chanos chanos* cultivated in ponds showed isometric growth patterns with values of 2.89-3.15 **(Biswas *et al.*, 2011)**. The value indicated an isometric growth pattern dermining that fish are able to maintain a constant body shape with proportional increase in length and weight. The lenght-weight relationship pattern showed relative growth, which means that the lenght-weight relationship pattern would be allowed to change according to time **(Jurniati *et al*. 2021).**

CONCLUSION

Chanos chanos stocked in pond waters can utilize most types of plankton that are available as food sources. The most consumed phytoplankton species were *Chaetoceros* sp. and *Nitzchia* sp., While the most consumed zooplankton were *Calanus* sp. and *Cyclop* sp. The proportion of zooplankton increased with the size of *Chanos chanos* and was found in the digestive tract of fish > 15 cm in length. The total growth and daily growth rate of *Chanos chanos* in ponds was very good and could reach the size of consumption within six months after stocking. In conclusion, the length and weight of *Chanos chanos* are directly proportional to the omega-3 content in the fish's body.

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