ISSN: 2008-8019 Vol 13, Issue 01, 2022



Fish Crop Rotation to Increase the Natural Food Base and Fish Productivity in Earthen Fish Ponds

Yaseen Hamed Farhan¹, Lydia M. Vasilyeva², Adelya Z. Anokhina³

¹Ph.D. student, Astrakhan State University, Russia, Ministry of Agriculture, Department of Agricultural Research, Baghdad, Iraq
ORCID: 0000-0002-8804-0837

²Ph.D in Agriculture, Professor, Chair of Biotechnology, Zoology, & Aquaculture, Astrakhan State University, 20a Tatischev Street, Astrakhan, 414056, Russia;

ORCID: 0000-0002-4927-2088

³Ph.D. in Biology, senior researcher Scientific and educational center "Sturgeon breeding", Astrakhan State University; Russia, 20A Tatishcheva str., Astrakhan, ORCID: 0000-0002-5470-3436;

Email: ¹yaseenrussia@gmail.com, ²bios94@mail.ru, ³yus-adehlya@yandex.ru

Abstract: The study was conducted in the south of the Russian Federation, to compare Fish crop rotation and its effect in natural food base and fish productivity. In the 2021 season, pond No. 3 was used for melon farming, while the remaining ponds 1.2.4 were used for fish farming, that in these three ponds was studied the extent of the effect of alternately culture on the natural food base as well as on the quality of fish in each pond, where pond No. 2 was used for fish farming immediately after planting watermelon, so used to compare with the other ponds, 1 and 4. Taking into consideration the research details, the present study examined the influence of natural food on the growth and productivity of fish, as well as limnological conditions in the polyculture system.

Keywords: Fish crop, natural food, fish productivity, fish ponds

1. INTRODUCTION

Plant and animal organisms play an important role in feeding pond fish, especially in the early stages of its development, when artificial food cannot replace natural. Earthen ponds are distinguished from any type of cultivation pond by the fact that fish live in habitat similar to their original habitat and depend on natural food in the water such as benthic animals and plankton, in addition to added feed. This system relies heavily on natural nutrition and may not need to any fertilizers, and if they are used in small quantities of organic fertilizers in order to develop natural food, this level may end up using some additional feed ingredients or feed of low nutritional value. This system is characterized by low costs and does not require much labor and also low risk (Ponomarev *et al.*, 2007).

Fish productivity is related to the primary production of phytoplankton, so the possibility of increasing it is of paramount importance to increase the productivity of the earthen ponds (Руденко 1986; Богданов,, 1991). Zooplankton organisms are particularly valuable and are food for the larvae and Young of all fish species as well as for many adult fish. They are very

ISSN: 2008-8019 Vol 13, Issue 01, 2022



small animals that are widely distributed, they live in ponds, rivers, lakes, reservoirs and other bodies of water, and they are food for many invertebrates and fish larvae. The natural food base of ponds is not a limited resource. Seasonal features of fish farming leave their mark on the individual processes of creating and developing the food base of ponds. Much in the periodic development of natural biomass depends on the technologies used to operate the ponds, as well as on the climatic zone of the location of the ponds. So in fish farming, in order to fully exploit the natural food supply and increase the productivity of ponds, joint farming of various species of fish is used. This method of co-cultivation of many objects is called "multi-culture" (Bikin, 1999). Multispecies culture is based on the co - culture of fish that feed on many food products-benthos's, plankton and detritus, which occupy different trophic levels in ponds (Vaseleva, 2000). The basis of feeding carp and other commercial fish (silver carp, grass carp) in the early stages of development (larva, fry, Fingerlings) is on small forms of phytoplankton and other food organisms. Common carp at the beginning of the growing season; they filter out zooplankton and consume carbohydrates to meet their energy needs, using exogenous enzymes derived from zooplankton (e.g., Cladocera) to digest carbohydrates. Silver carp is a filtration feeder equipped with a specialized device capable of filtering micron-sized particles. The gills merge into a reticular filter, and the epithelial organ secretes mucus, which contributes to the destruction of small particles. A powerful pump inside the mouth pushes water through this filter. Silver carp does not have a stomach, and small fish feed on zooplankton as the main food. The basis of food, both white carp and bighead carp can reach 82-84.0% of phytoplankton. The proportion of zooplankton in silver carp reaches 14% (Кружилина, 2002). Live foods contain proteins, fats and whole carbohydrates. They are a source of essential amino acids (Касаткина и щербина 1999). In conditions of high-density farming, animal food already increases its appetite by 0.1% of the mass of fish, activates digestion and assimilation of additional feed, which reduces feed costs (Щербина и др). 1992). In order to provide a natural food base in the earthen ponds, alternation between agricultural crops and fish can be used. So in the last ten years, the term "organic aquaculture" practice has appeared which includes the principle of the intersection of two ascending global practices; aquaculture and organic production or the so-called (Fish rotation). The latter is expressed in the refusal to use pesticides, chemical fertilizers, antibiotics, hormones, etc. (Лагуткина и пономарёв 2018).

2. MATERIALS AND METHODS

The study was conducted in the south of the Russian Federation, specifically at the BAM farm in Astrakhan province, where the research plan was developed using four ponds 1, 2, 3 and 4, which have an area of 25, 25, 30 and 30 hectares, respectively. In the 2021 season, pond No. 3 was used for melon farming, while the remaining ponds 1.2.4 were used for fish farming, that in these three ponds the effect of culture was studied alternately on the quality of fish in each pond, where pond No. 2 was used for fish farming immediately after watermelon cultivation, so it was used to compare with the other ponds 1 and 4. In mid-March, the fish were brought from the fry ponds of the farm and distributed to the feeding ponds, where they were raised to reach commercial weight. Fish are weighed and distributed according to the following proportions (Common carp 40%, Gras carp 20% and silver carp 40%). In pond No. 1, 12,000 common Carp at an average weight of 46 grams plus 6600 Grass carp with an average weight of 30 grams, and 12,100 silver carp with an average weight of 26 grams. In the pond No. 2 it were placed 12000 common carp with an average weight of 40 grams, 6300 grass carp with an average weight of 38 grams, plus 12,900 silver

ISSN: 2008-8019 Vol 13, Issue 01, 2022



carp with an average weight of 30 grams. 14,100 common carp were placed with an average weight of 40 grams, 6100 grass carp with an average weight of 38 grams and 13,500 silver carp with an average weight of 30 grams. In pond No. 4, after the end of the season, the ponds were prepared and equips for the winter. Fish weight measurements were taken every two weeks with registering other indicators like oxygen, temperature and PH. Multi-read Portable Water Analyzer type (hi 9829-01042) are used to measure dissolved oxygen, pH and temperature. Green feed was given such as cane and reeds, as well as cereals such as wheat and barley by 5% of the body weight. The fish were fed manually once a day at ten o'clock in the morning, where special places were allocated for food called (Feeding points), and each pond contains six feeding places where the feed was given (grinding wheat and Barley).

As for the method of estimating the amount of phytoplankton, zooplankton and benthos in ponds: to assess the food base of ponds, samples of plankton were taken once at the beginning and end of the growing season in March and September in 2021. The ponds used in the study are numbered from 1 to 4 and are earthen ponds with a clay substrate of 30-40 mm. The water level in the ponds fluctuates throughout the year from 1 to 1.8 meters. Studies of phytoplankton in ponds, including the determination of quantitative indicators of algae (abundance and biomass) were carried out monthly from May to September. Collection and processing of algal material was carried out according to the Kuzmin method (1975), generally accepted in aquaculture. The most common method of phytoplankton concentration is sedimentation (Sorokin, 1979). A sample of water intended for plankton thickening was collected using plankton Apshtein net (mesh size 25 micrometers, collection cup capacity of the net-200 ml). Using a microscope, we counted all the phytoplankton present in the cell, moving it horizontally and vertically. The number of cells was used in the reverse calculation of cell density, where the density of plankton is estimated from the average number of phytoplankton, which was recorded and expressed numerically per liter of aquarium water. Phytoplankton was calculated according to the following formula.

 $N = n \times v / V$.

Where N = total number of objects per liter of filtered water

n = total number of organisms counted in a 1 ml plankton sample

v = concentrated plankton sample volume (ML)

V = Volume of all filtered water (50 liters).

Zooplankton was collected at the site using a zooplankton net (diameter 20 cm, mesh 34 μ m) lowered 2 m vertically in four different places of the pond. Then contents of the net cup were preserved with 4% buffer formalin until laboratory processing.

All samples were processed and analyzed in the laboratory (International Technical Laboratory, Baghdad, Iraq).

Zooplankton samples were examined on a reticular petri dish under a dissection microscope. Samples were identified as rotifers, Cladocera and copepod and other varieties were counted to obtain and calculated to get the number of organisms /L. The subsample was digitalized to determine the conversion of zooplankton to raw weight (Dumont *et al.* 1975; Bottrell *et al.* 1976; Bowen 1996). The qualitative composition was studied in accordance with generally accepted determinants, quantitative zooplankton by using the masses of organisms according to Mordukhai-Boltovek (1954).

In selection of benthic samples, a Peterson dredger was used according to the scheme of four sampling points. Zoobenthos samples were processed according to generally accepted methods (Abakumov, 1983). Species identification was carried out using the "Atlas of Invertebrates of the Caspian Sea" (Birstein, 1968) and the "guide of freshwater invertebrates

ISSN: 2008-8019 Vol 13, Issue 01, 2022



..." (Kutikova, 1977). The number and biomass of species and forms were determined per m2 of the bottom surface: number (specimen/m2), biomass (gr/m2).

3. RESULTS AND DISCUSSION

In Table (1), which shows the results of the readings of environmental factors affecting the growth and life of fish, where in pond No. 1 the pH level ranged throughout the study period between 7-8.2, while in pond No. 2 it was between 6.5 and 7.8 degrees, it was in the narrowest range in pond No. 4: 6.8 - 7.7. The temperature in the three basins recorded the lowest temperature in March where it was 6.5 °C at the beginning of the experiment in ponds 2 and 4 and began to increase until the highest degree reached 31 °C in August in pond 1 and in basins 2 and 4 did not exceed 29 and 29.5 degrees Celsius respectively in August. The best water temperatures were in May, June and mid – July, with temperatures ranging between 23-28 in all ponds. The dissolved oxygen content was reduced to 4.6 mg / 1 in August, the remaining months during the experiment ranged from (5.1-9) mg / 1. In ponds 1 and 4, the oxygen content of ponds water ranged from 5 to 7.6 mg / L to 4.8 to 8 mg / L during the study period.

Table (1)

Indicators	Pond 1				d 2 after Meloi	1	Pond 4		
Months	P H	Temperatur e °C	DO. mg/L	P H	Temperatur e °C	DO. mg/L	P H	Temperatur e °C	DO. mg/L
March	8	7	9	7. 5	6.5	7.6	7. 4	6.5	8
April	8. 2	14	8.6	7. 8	13	7	7. 5	12.5	7.9
May	7. 7	24	7.2	7. 2	23	7	7. 3	23	7.6
June	7. 5	26.5	7	6. 5	25	7.2	6. 8	25.5	6.8
July	7. 6	29	5.1	6. 9	28	6	7	28	6
August	7	31	4.6	7	29	5	7. 2	29.5	4.8
Septembe r	7. 5	22.5	6.7	7. 5	21.5	6.5	7. 7	20	6

The results of analysis of study ponds hydrobiology, showed that pond No. 1 in the beginning of the season (April) before the farming of fish , it contains 2473200 cells \ L of phytoplankton which contain several groups, the most important of which were (Bacillar, Chlorophyta, Cyanophyta, Euglena). As well as 54960 ind./ M^3 of zooplankton, the weight of which were 2.2006 g/ M^3 , the most important groups were (Copepoda, Cladocera, Rotifera). It also contains the zoobenthos groups, which contains many orders, of them (Insecta Notonecta, Chironomidae, Oligochaeta, Tubifex spp., Limnodrilus sp, Mollusca, and Physa). Zoobentus groups were 1605 ind M^2 and weighing 7.08 gr/ M^2 . At the end of the season in

ISSN: 2008-8019 Vol 13, Issue 01, 2022



September, the number of phytoplankton was 2,041,000 cells/L. Group of zooplankton reaching to 71165 ind/m3 and weight to 1.189 gr/m3. The number of zoobenthos groups reached 1412 ind./m² and a weight of 5.94 gr/m².

In pond No. 2, which was cultured with fish immediately after the cultivation of melones , the results of hydrobiological analysis at the beginning of the season in April showed that it contains 6920600 cells / L. phytoplankton, and 144810 ind/m3 of zooplankton at the weight of 4.909 gr/m3. As well as 3415 ind/m2 of zoobenthos this was weighing 13.4 gr/m2. The results of the analysis at the end of the season in September showed that the pond No. 2 contains 2.946,200 cells / L. phytoplankton and 126349 ind/m3 zooplankton which were weighing 2.449 g/m3. This pond was containing 1747 inds/m2 zoobenthos that reached a weight of 10.46 gr/m2.

In pond No. 4, the analysis carried out at the beginning of the season before Fish Culture showed that it contains 2,897,000 cells/ L phytoplankton , and also the 54962 inds/m3 of zooplankton which weighing 1.831 g/m3 , as well as the pond contains 304 inds/m2 zoobenthos, which was weighing 2.02 g/m2 . The results of the analysis in the month of September end of the season showed that pond No. 4 contains 2,484,000 cells / L. phytoplankton, and also on 68511 inds/m3 zooplankton which reach an 1.066 gr/m3 , as well as the it contain 156 inds/m2 zoobenthos with a weight reached 3.31 gr/m2 .

Table (2) clearly demonstrates the general average of numbers and biomass to facilitate the observation of the effect of fish culture alternately with the melons where we observed a clear increase in numbers and biomass of phyto and zooplankton in the pond that was farmed with fish directly after the melons (pond 2). The phytoplankton here increased by 157.8%, the zooplankton increased to 161.7% and the zoobenthos by 257.8% before the start of the fish culture season, compared with ponds 1 and 4. After the fish culture season we noticed that the number of phytoplankton in pond 2 increased by 30.2% in comparison with ponds 1 and 4. Whereas Zooplankton increased by 80.9%, while zoobenthos increased by 122.8 %.

Table (2)

Type of ponds	Plankton and	At the beging growing seasor	ning of the (March)	at the end of the growing season (September)		
	benthos	Number	biomass	number	biomass	
Ponds without melons Pond No. 1	Phytoplankton Cells/ L	2685100	-	2262500	-	
and Pond No.	Zooplankton Ins /m3 - g/m3	54961	2.0158	69838	1.1275	
(Average total) 2021	zoobenthos ins./m2 - g/m2	954.5	4.55	784	4.625	
Pond after	Phytoplankton Cells/ L	6920600	-	2946200	-	
Pond after melons Pond No. 2 2021	Zooplankton Ins /m3 - g/m3	143810	4.909	126349	2.449	
110. 2 2021	zoobenthos ind./m2 - g/m2	3415	13.4	1747	10.46	

ISSN: 2008-8019 Vol 13, Issue 01, 2022



From the results of the monthly weight of fish we observed that, in April the superiority fishes of pond No. 2 by weight from the rest of the study ponds; where the average monthly weight of common carp was 126 gr, 120 gr for grass carp, and 116 gr for silver carp. At the same time in pond No. 1 the monthly weight average of common carp was 90 gr, 98 gr for grass carp and 92 for silver carp. In pond No 4 the average monthly weight of common carp was 105 gr, grass carp was 98 gr, and silver carp was 95 gr. (Table 3). This superiority in weight is due to the presence of health conditions and the abundance of Natural food base that gave better growth potential in pond No. 2 than other ponds. This increasing result is consistent with Rudenko (1986) and Bogdanov (1991) about that the fish productivity is related to the primary production of plankton. So the possibility of increasing primary production is of great importance to increase the productivity of the fish ponds. At the end of the harvest season in mid-September, the average final weight of fish in pond No. 1 were as following: 503 ± 12.06 gr for common carp, 533 ± 12.86 gr, for grass carp, and 520 ± 15.44 gr for silver carp. In pond No 2, the average monthly final weight was 684 ±13.87 gr for common carp (36% higher than of pond No 1 and 33.6% higher than in pond No 4). Regarding grass carp, it was 650 ± 17.92 gr, (the highest final weight rate of pond No. 1 by 21.9% and higher than pond No. 4 by 22.6 %). For silver carp it was 677 \pm 18.58 gr (highest final weight rate from pond No. 1 by 30.2% and higher than pond No. 4 by 24 %). As for pond No. 4, the final average weight was 512 ± 9.75 gr for common carp, and for grass carp was 530±13.56 gr, and for silver carp was 546± 13.39 gr. When analyzing the data statistically, we found significant differences between ponds at a significant level (p>0.01) in all monthly weights (Table 3).

It should be mentioned that these big increases were due to the rich natural dietary base which providing main important nutrients that fish need for growth and activity. So, this increase occurred and is consistent with what Bogatova (1992) found. The food base of the ponds should contain the required proportion of natural biologically active substances (amino acids, enzymes) enhances their appetite, activates the digestion and assimilation of compound feed, reducing feed costs. Only in this case one can count on the active growth of Fish and the increase in quality indicators of individuals.

Table (3)

				1 4010	(-)						
Ponds	Average weight / gr. 2021										
lonus			Pond 1	Pone	d 2 after	melons	Pond 4				
Months	Comm on Carp	Grass carp	Silver carp	Comm on Carp	Grass carp	Silver carp	Comm on Carp	Grass carp	Silver carp		
March	46	30	26	40	38	30	40	38	30		
April	90 ±5.24b	98 ±5.70 b	92 ±3.73 b	126 ±6.83a	120 ±7.06 a	116 ±5.73 a	105 ±5.87b	98 ±5.66 b	95 ±4.08 b		
May	210 ±5.60c	225 ±7.62 b	219 ±5.32 b	270 ±6.19a	275 ±7.52 a	255 ±7.57 a	230 ±4.91b	205 ±4.83 b	212 ±6.39 b		
June	320 ±9.06c	360 ±8.21 b	350 ±9.40 b	435 ±10.60 a	442 ±9.97 a	438 ±10.3 4a	355 ±9.38b	345 ±10.2 2b	352 ±8.63 b		
July	390	425	413	535	545	539	410	430	445		

ISSN: 2008-8019 Vol 13, Issue 01, 2022



	±8.60b	±10.9	±9.35	±23.13	±12.0	±11.2	±6.45b	±7.92	±22.1
		4b	c	a	7a	2a		b	3 b
August	460	495	485	620	605	602	480	495	505
	±12.21	±11.7	±13.9	±15.95	±15.8	±16.3	±11.43	±11.6	±19.1
	b	8b	6b	a	9a	3a	b	3 b	3 b
Septemb er	503	533	520	684	650	677	<i>E</i> 10	530	546
	±12.06	±12.8	±15.4	±13.87	±17.9	±18.5	512	±13.5	±13.3
	b	6b	4b	a	2a	8a	±9.75b	6b	9b

4. CONCLUSION

Due to the key role of plankton in fish ponds, there is a continuous need to study the effect of their abundance and biomass on the nature of fish growth.

The number of phytoplankton aggregates increased by 157.7%, the zooplankton aggregates increased by 161.7%, the zoobenthos aggregates increased by 257.8%. Before the start of the breeding season (fish farming), the number of phytoplankton aggregates increased by 30.2%, the zooplankton aggregates increased by 80.9%, and the zoobenthos increased by 122.8 %.

This increase in the food base led to an increase in the fish product, it was noted that pond No. 2 was the best where the final weight was 684 ± 13.87 gr for common carp (where it exceeded pond No. 1 by 36% and pond No. 4 by 33.6%). Also, the final weight of grass carp was 650 ± 17.92 gr (is outperforming pond No. 1 by 21.9% and pond No. 4 by 22.6%). Also, the final weight of 677 ± 18.58 gr for silver carp (the highest final weight rate of pond No. 1 by 30.2% and higher than pond No. 4 by 24%).

When analyzing the data statistically, we found significant differences between ponds at a significant level (p>0.01) in all monthly weights where pond number 2 outweighs ponds 1 and 4.

5. REFERENCES

- [1] Ponomarev S. V., Lagutkina L. Yu., Kireeva I. Yu. 2007. Farm aquaculture: Recommendations. M.: FGNU "Rosinformagrotech", 192 p. (In Russian)
- [2] Bottrell, H.H., Duncan, A., Gliwicz, Z., Grygierek, E., Herzig, A., Hilbricht-Ilkowska, A., Kurasawa, H., Larsson, P., Weglenska, T., 1976. Review of some problems in zooplankton production studies. Nor. J. Zool. 21, 477–483.
- [3] Dumont, H.J., Van de Velde, I., Dumont, S., 1975. The dry weight estimate of biomass in a selection of Cladocera, Copepoda and Rotifera from the plankton, periphyton and benthos of continental waters. Oecologia 19, 75–97. https://doi.org/10.1007/BF00377592
- [4] Vinberg. G.G., 1966. "Skorost rosta Iintensivnost obmena u zhivotnykh." /Growth rate and metabolic intensity in animals/. "Uspekhi sovremennoy biologii" /Achievements in contemporary biology/ Vol. 61, No.2, pp. 274 293.
- [5] Zaika, Viktor E (1972): (Table 1) Abundance of microzooplankton in surface waters of the Mediterranean Sea in August 1970. PANGAEA, https://doi.org/10.1594/PANGAEA.754792.
- [6] Abakumov V.A. 1992. Guide to hydrobiological monitoring of freshwater ecosystems St. Petersburg: Hydrometeoizdat, 1992. 246 p.

ISSN: 2008-8019 Vol 13, Issue 01, 2022



- [7] Bekin A.G. Universal fish pond. // Fish farming and fishing. 1999, No. 2. pp. 10-11.
- [8] Birstein, Ya. A. 1968. Atlas of invertebrates of the Caspian Sea / Moscow, Food industry, 1968, 415pp.
- [9] Bogatova I.B. 1992. Industrial breeding of branchous crustaceans in the USSR// Modern problems of studying branchous crustaceans. -St. Petersburg: Hydrometeoizdat, pp. 122 129.
- [10] Bogdanov N.I., Komilov F.S., Yunusov M.K., Egamov M.S. 1991. Mathematical modeling of a managed highly productive ecosystem of a fish pond // Scientific Academy of Rep. Tajikistan press. Department of Biological Sciences. No. 1 (122). pp. 14-18.
- [11] Vaseleva L. M. 2000. Biological and technological distinctiveness commercial aquaculture in the lower Volga region. Astrakhan, 190 p.
- [12] Kasatkina A.E., Shcherbina M.A. 1999. Metabolism in carp in the embryonic and early postembryonic periods //Questions of physiology and feeding of fish: Collection of scientific works /VNIIPRH. Moscow. Issue 74: 3-26.
- [13] Kuzmin G.V. 1975. Phytoplankton. Species composition and abundance // Methodology for studying biogeocenoses of inland reservoirs. M.: Nauka, pp. 73-87.
- [14] Kutikova L. A., Starobogatov Ya. I. (ed.). 1977. Determinant of freshwater invertebrates of the European part of the USSR (plankton and benthos). Hydrometeoizdat, Leningrad.
- [15] Lagutkina L.Yu., Ponomarev S.V. 2018. Organic Aquaculture as a promising direction for the development of fisheries industry (review). Agricultural biology. Animal biology. Volume: 53 Number: 2.
- [16] Mordukhai-Boltovsky FD. 1954. Materials on the average weight of aquatic invertebrates of the Don basin//Tr. prob. and temat. meeting. ZIN. No. 2. -pp. 223-241.
- [17] Rudenko, G.P. 1986. Production and hydrobiological studies in inland reservoirs: collection. Lenigrad: Promrybvod, issue 252, pp. 45-50.
- [18] Sorokin Yu. I. 1979. To the method of concentrating phytoplankton samples // Hydrobiol. journal. Vol. 15, (2) pp. 71-76.
- [19] Shcherbina M.A., Kiselev A.Yu., Kasatkina A.E. 1992. Growing carp in ponds. Minsk: Urajay, 136 p.
- [20] Kruzhilinas.I. N. 2002. Feeding of plant-eating fish from the bottom of the basin of the Sakhalin salt of the Kremenchug water storage. Theses of the proceedings of the XIII Conference of young scientists "Biology of the inner water: problems of ecology and biodiversity", Borok.
- [21] Lambert Niyoyitungiye, Anirudha Giri, Bhanu Prakash Mishra. 2020. Quantitative and qualitative analysis of phytoplankton population in relation to environmental factors at the targeted sampling stations on the Burundian littoral of Lake Tanganyika. International Journal of Fisheries and Aquatic Studies, AkiNik Publications 8 (1, Part B), pp.110-121.
- [22] APHA. 1995. Standard methods for the examination of water and wastewater. American Public Health Association, Washington, USA
- [23] Patil S. S. and More V. R. 2017. Zooplankton sampling methods in freshwater lakes. Rev Res. 6 (12):1–9.