Comparison of different nitrogen compounds in three different environments of the Gwatar shrimp farms complex in the Gwatar Gulf region (Baluchestan-Iran)

Khodami S.[1*]; Attaran-Fariman G.[2]; Ghasemzadeh J.[2]; Mortazavi M. S.[3]

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Abstract

This study aimed to investigate the quality of effluent water of a large shrimp farming complex in Chabahar region in Sistan and Baluchestan province (Iran). Fifteen sampling stations were designated in three distinct areas of these farms; i.e. the water supply canal, the drainage canal and the receiving area of the farm effluents in Gwatar Gulf. Water samples were taken from June to December 2004 for measurement and monitoring of nitrate, nitrite and ammonia compounds. The results revealed that the shrimp farms effluents have a profound effect on the water quality and aquatic environment of the drainage canal and the Gwatar Gulf. The average concentration of nitrate, nitrite and ammonia remained considerably higher in the drainage canal and Gwatar Gulf compared to the supply canal throughout the study. The amount of these compounds increased considerably in the drainage canal during the final stages of culture period and in Gwatar Gulf at post monsoon. The results of one way analysis of variance (at $P<0.01$ level) showed that all physical and chemical factors except water temperature were significantly different between stations. Considering that more than 25% of the shrimp farms of this complex were active during this study the measured values of nitrate, nitrite and ammonia compounds in the effluents were at the optimum range recommended for the shrimp farms.

Keywords: Aquaculture, Shrimp farming, Effluent, Monsoon, Nutrient, Gwatar, Environmental impact

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1-Iranian Fisheries Research Organization, P.O. Box: 14155-6116, Tehran, Iran.
2-Faculty of Marine Science, Chabahar Maritime and Marine Science University, Chabahar, Iran.
3-Persian Gulf and Oman Sea Ecological Research Institute, Bandar Abbas, Iran.
Corresponding author’s email: Shkhodami@yahoo.com

Introduction

Shrimp farming has been one of the traditionally important activities in tropical and subtropical coastal waters with a worldwide rapid growth in the last decades of the last century. Currently more than 70% of the total world shrimp production, is harvested in south East Asia and Central America, most of which is exported to developed countries (Shimoda, et al., 2007; Kakoolaki et al., 2010). However the shrimp aquaculture industry is facing extraordinary criticism for unsustainable practices (Naylor et al., 1998). Shrimp farm can impose various environmental impacts in water quality of the receiving water bodies. The uneaten feed and excreted waste have created water quality problems (Wetzel, 2000; Boyd and Green, 2002; Alonso-Rodriguez and Paez-Osuna, 2003;). The effluents from shrimp farms cause nutrient enrichment in recipient environment that result in high phytoplankton density, oxygen depletion, increase in suspension solids, phosphorous and nitrogen compounds, and decrease in light penetration, and also eutrophication of coastal water bodies. This can change the benthic macrofauna, composition of other fauna and flora, (Roonbcak, 2001; Rey, 2002; Boyd 2003), damage the mangrove and wetland habitats and deplete the world fish stocks (Rivera-Monroy, et al., 1999; Paez-Osuna, 2001; Pereston, 2002; Alonso-Rodriguez and Paez-Osuna, 2003). Intensive shrimp aquaculture is regarded as an incompetent production system because only approximately 24% of the nitrogen of the feed input is converted into the shrimp harvested, and the rest is finally discharged into the surrounding environment. Research on the measurement, monitoring and interaction of different chemical and biological factors of water in shrimp farms and their effluents have been carried out in different countries such as; Honculada (2000) in the Philippines, Dierberg et al. (1996) and Tookwinas and Songsang-jnda (1999) in Thailand, and Jones et al. (2001) in Australia.

Considering shrimp farming as a new and improving aquaculture practice in southern coastal parts of Iran, it faces some critical and challenging problems in terms of water supply and monitoring water quality. The environmental effects of shrimp effluents on the receiving water bodies (mainly coastal waters of Persian Gulf and Oman Sea) especially after the outbreak of white spot disease and recent red tide and fish death incidents had drawn the attention of many researchers in recent years. Omidi (2003) studied the environmental effects of shrimp farms effluents in the Helleh and Delvar region of Bushehr province in southwest of Iran. In Hormozgan province located in the Persian Gulf coast line, Akbarzadeh (2003) investigated the water quality of shrimp farm effluents in Tiab area, and Khodami (2005) conducted a similar research in Gwatar shrimp farms in southeast of Iran. This study aimed to research the trends of nitrogen compounds variations in the water source and its subordinate supply canals, the effluents in drainage canal and its receiving water body in Gwatar Gulf, Chabahar region, Sistan and Baluchestan province in south east coast of Iran.

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Materials and methods

This study was conducted at the Gwatar shrimp farming complex during the course of one culture cycle from June to December 2004. This complex is located at 100 km east of Chabahar along the coastal waters of Gwatar Gulf which is joint to the Oman Sea, and covers an area of approximately 4000 hectares of shrimp farms which are operated by a cooperative body of shrimp farmers. The project was financed and supported by Iran Fisheries Research Organization. A total of 15 sampling stations were selected, of which 2 stations were in the water supply canal, 8 stations along the drainage canal and 5 stations in the Gwatar Gulf (Figure1).

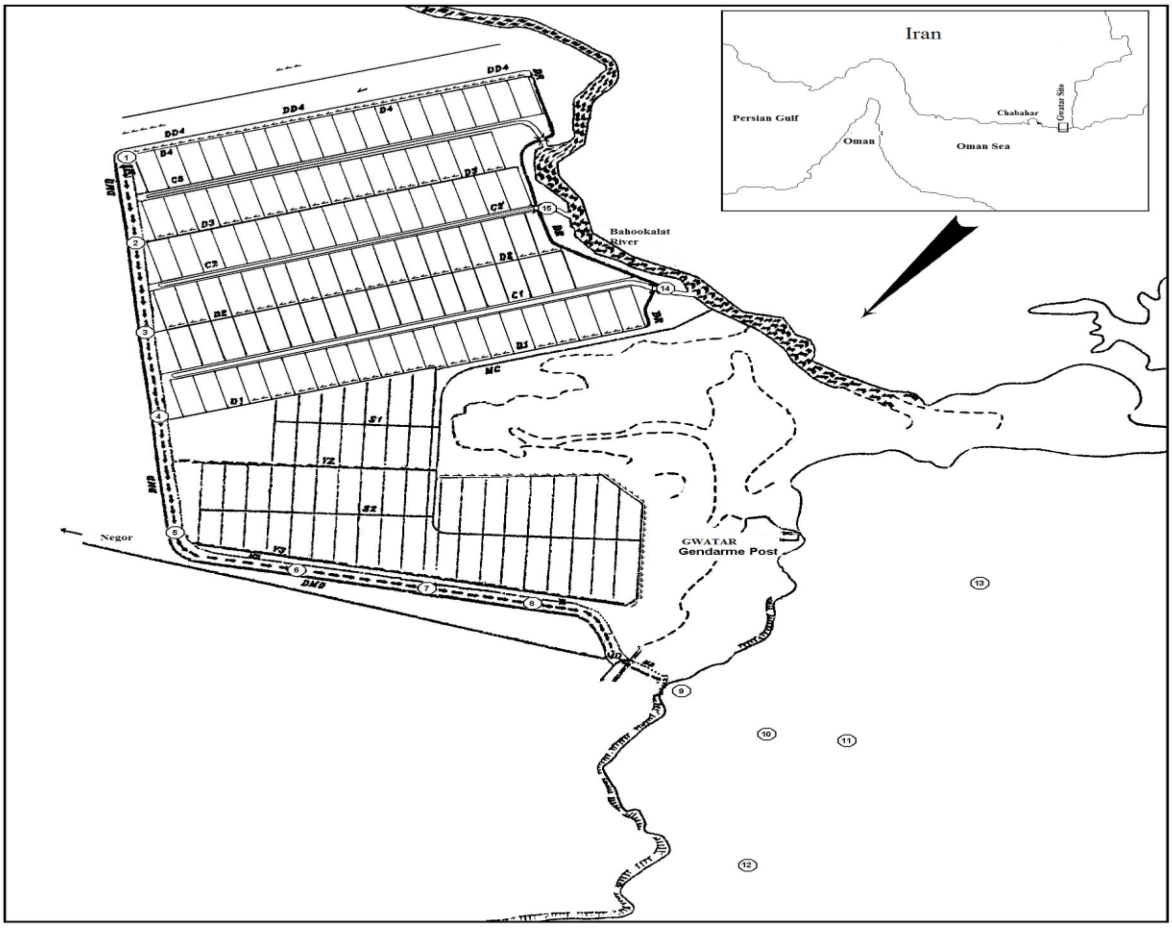

Figure 1: Gwatar shrimp farm complex and sampling stations.

Water samples were taken twice a month by Rutner's bottle, and transferred to the laboratory at 4°C. At the same time the water temperature and pH were also recorded. The samples were filtered by a vacuum pump and Millipore filter paper with a 47mm diameter and 0.45 micron size. The filtered samples were used to measure different forms of nitrogen compounds. Nitrite, nitrate and ammonia were measured based on Grassoff et al. (1983) method and their absorbance were measured by a spectrophotometer Hitachi model U-2000 (MOOPAM, 1999). The determination of nitrite is based on the reaction of nitrite with an aromatic (sulphanilamide) which leads to the formation of a diazonium compound. This diazo compound then couples with a second aromatic amine N-(1-naphtyl)-ethylenediamine to form the azo dyo. The absorbance was measured at 540 nm by a spectrophotometer. Determination of nitrate is based on nitrate reduction (cadmium) to nitrite which is then determined colorimetrically via the formation of an azo dye. The absorbance was measured at 540nm by a spectrophotometer. The amount of ammonia was measured on the formation of the blue colored indo phenol by phenol and hypochlorine. The absorbance was measured at 630nm by spectrophotometer (MOOPAM, 1999). Salinity determination is based on the titrimetric method and the amount of salinity with use of cholorinity was determined (MOOPAM, 1999).

Results

The nitrate variations at all of the stations during June to December 2004 are shown in Fig. 2. The mean nitrate level was (0.265±0.007 mg/l). The minimum level of nitrate (0.14 mg) was recorded at station 13 in the Gwatar Gulf during the first half of September, and the maximum content (0.759 mg/l) was measured at station 7 in drainage canal in November.

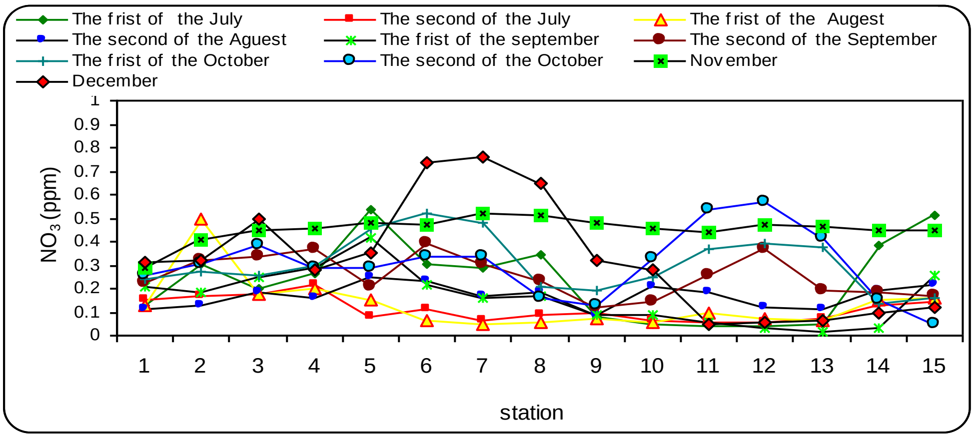

Figure 2: Nitrate variation at different sampling stations from the 1st half of July to December

The average monthly variation of nitrate is shown in Figure 3. The average monthly nitrate in the drainage canal and Gwatar Gulf had a gradual increasing trend in the course of study. This increase was remarkable in the drainage canal during the second half of the culture period (September to November), and after the monsoon in the Gwatar Gulf. The average monthly nitrate variation in the drainage canal stations were higher compared to the supply canal and the Gwatar Gulf stations. The average nitrate variation in the stations shows the amount of nitrate is increasing along the drainage canal from its inlet towards the outlet with a maximum of (0.340 ±0.017mg/l) at station 6.

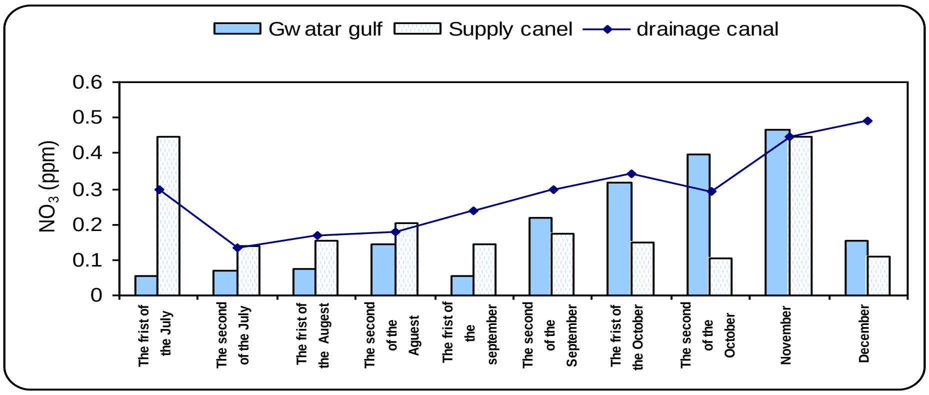

Figure 3: The mean monthly nitrate variation during the study period from July to December

Fig. 4 shows the nitrite variations throughout this study which was often higher in drainage canal compared to the supply canal and Gwatar Gulf during the first half of the culture period. This trend continued until late October with a sharp increase in nitrite level at station 11 in Gwatar Gulf. The average nitrite content in this area was (0.014±0.001 mg/l). The minimum level of 0.001 mg/l was recorded at several stations in early July, while the maximum level was 0.072 mg/l at station 11 in late October.

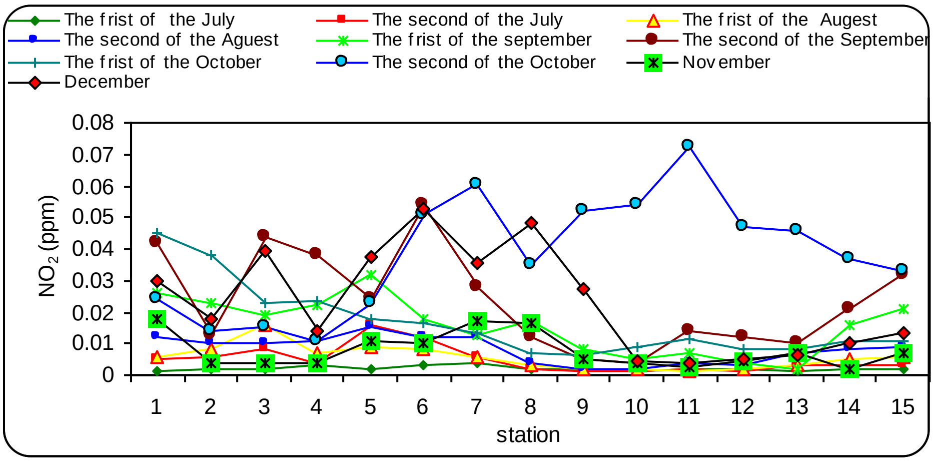

Figure 4: Nitrite variation at different sampling stations from the 1st half of July to December

The average monthly variation of nitrite is shown in Fig. 5. The average monthly nitrite variation in the drainage canal stations were higher compared to the supply canal and the Gwatar Gulf. The maximum monthly nitrite is after the monsoon in Gwatar gulf and supply canal (the second of October).

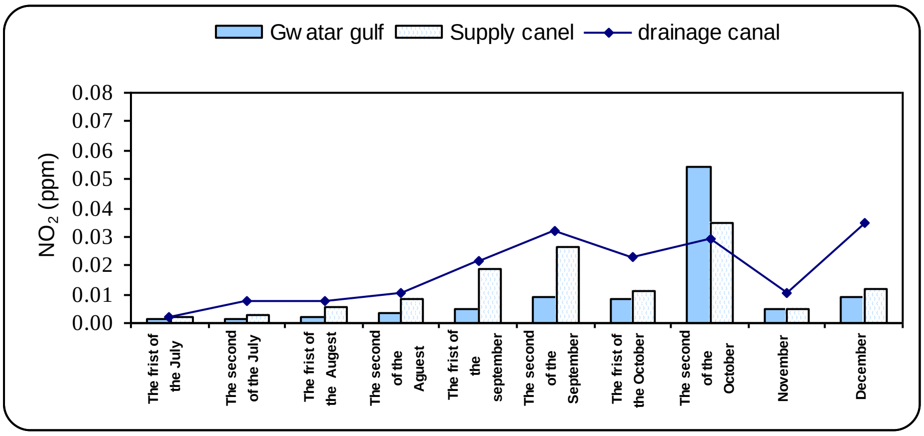

Figure 5: The average monthly nitrite variation during the study period from July to December

The variations of ammonia concentration at all stations are shown in Fig. 6. The ammonia content in the drainage canal was always higher than those of supply canal and Gwatar Gulf. The average concentration of ammonia was 0.072±0.003 mg/l, with a minimum of 0.001 mg/l recorded at stations 9 and 13 and a maximum of 0.329 mg/l measured at station 6. The ammonia variation in the Gwatar Gulf followed almost a similar pattern as in the drainage canal, but it was different from the supply canal.

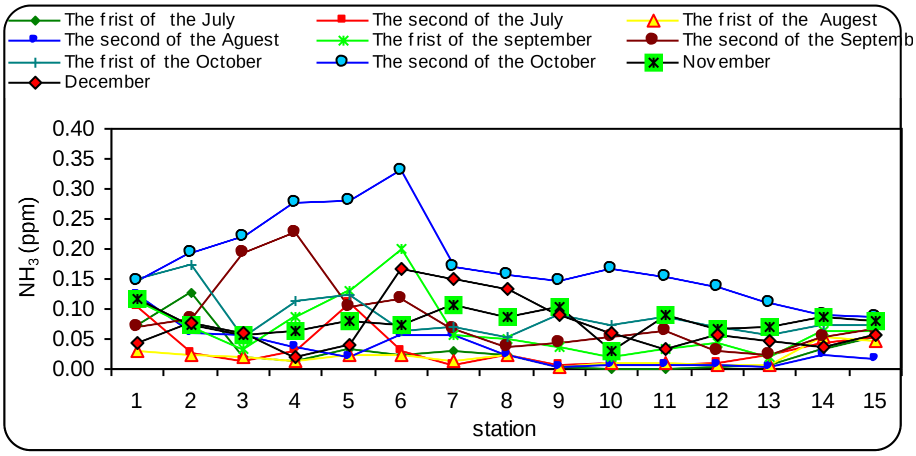

Figure 6: Ammonia variation at different sampling stations from the 1st half of July to December

Fig. 7 shows the average ammonia variation in the sampling stations. According to this figure the amount of ammonia is increasing along the drainage canal from its inlet towards the outlet with a maximum of 0.108±0.017 mg/l at station 6. After this station the ammonia concentration declines with a minimum of 0.037±0.006 mg/l at station 13 in the Gwatar Gulf.

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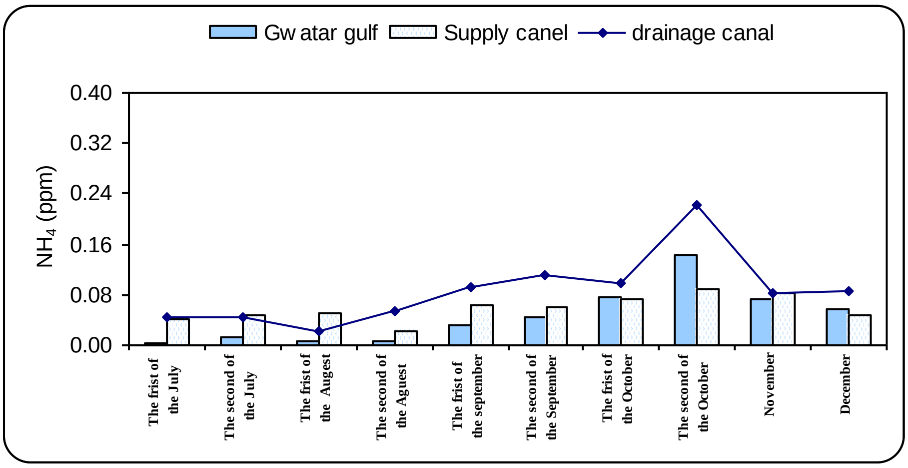

Figure 7: The average monthly ammonia variation during the study period from July to December

Table1: Results of a Duncan' new multiple range test to determine the month can be classified in one group

Parameter	Region S	G	D	order
NO_3	*			SO <De <SJ <FS <FO <FA <SS <SA <N < FJ
		*		FJ< FS< SJ< FA< SA< SS< De< FO< SO< N
			*	SO <SA <SJ <FA <FS <FJ <SS <SO <N< De
NO_2	*			N≤ FO< De< FA< FS< SS< SJ< SO< FJ< SA
		*		N< FS< FJ< SJ< FO< SS< De< FA< SA< SO
			*	FJ≤ SJ ≤FA≤ N< SA< FS < FO< SO< SS< De
NH_3	*			SA< FJ<De < SJ< FA< SS< FS< FO< N< SO
		*		FJ < SA< FA < SJ< FS< SS < De < N< FO < SO
			*	FA< SJ < FJ < SA< N < De < FS < FO < SS < SO

The cases which are underlined with a common line indicate mean values are not significantly different (Duncan' s new multiple rang test , (P≤ 0.01). S: Supply canal ;G: Gwatar Gulf ;D: Drainage canal ; De :December ;N: November; SO: The second half of October; FO: The first half of October; SS: The second half of September; FS: The first half of September ;SA: The second half of August; FA: The first half of August; SJ: The second half of July; FJ: The first half of July

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Table2: Results of a Duncan' new multiple range test to determine the stations can be classified in one group

Parameter	Order
NO_3	9< 13<14< 10< 1< 11< 12< 15<8< 4< 3< 2<5<7< 6
NO_2	10< 13<12< 14< 9< 11< 2< 4< 15< 8< 3< 5<7< 1< 6
NH_4	13< 12<10< 11< 9< 8< 7< 15< 14< 3< 4< 2< 5< 1< 6

Results from the one way analysis of variance for comparing the average of each of these parameters (nitrite, nitrate and ammonia) at all stations showed a significant difference (at P<0.01 level). Analysis of Duncan test for the similar stations showed that the stations in the Gwatar Gulf and one way analysis of variance for comparison of monthly average of the nitrite, nitrate and ammonia showed that there is a significant difference between the monthly average of these factors (P<0.01).

Discussion

Nitrate is a nitrogenous compound which is considered as one of the nutrients in sea waters and has the least toxicity to aquatic animals even in large concentrations (Boyd and Clay, 1998). Nitrate is the terminal product of nitrification. The optimum level of nitrate at 96h LC_{50} for marine organisms is expressed as 1000ppm (Tomasso and Carmichael, 1986; Price and Morel 1994). The Australian and New Zealand Environment and Conservation Council (ANZECC, 1992), has recommended that the nitrate nitrogen concentration in coastal waters should not exceed 0.005 mg/l. In this study the higher nitrate level in drainage canal compared to the Gwatar Gulf especially in the first stages of shrimp culture is mainly due to the use of nitrogen fertilizers (urea and nitrate fertilizers). Fertilizers can increase the nitrogen level in pond water which is gradually discharged into drainage canal and adjacent coastal ecosystems by flushing, water exchange and water circulation (Mortazavi, 1999). The average nitrate level in the drainage canal of this study area reported by Khodami (2005) in 2001 and 2002 were 0.350±0.017 ppm and 0.349+0.024 ppm respectively. Comparison of these figures with our results shows a lower nitrate concentration in the latter. This seems to be due to a decrease in the culture area in our case and conversion of different nitrogen

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forms into each other in the drainage canals.

In a similar study in the shrimp farms at Tiab of Hormozgan province the highest nitrate level was reported in the effluents discharged into the drainage canals, compared to the supply canal and effluent receiving areas (Akbarzadeh, 2003). The nitrate levels of the northern and southern sites of this project were reported as 0.572±0.44 ppm and 0.502±0.323 ppm respectively. In Iran there are not any regulations on aquaculture effluent water quality requirements set or recommended by the government. The only published and available regulation is about the agricultural waste water effluents, developed by the Iranian Environmental Protection Organization (IEPO). However the nitrate concentrations measured by different researchers show that it was much less than 50 ppm which is determined by IEPO for the agricultural effluents. The average monthly level of nitrate in Gwatar Gulf is increased after monsoon (Fig. 4) which can be attributed to the upwelling environmental changes induced by monsoon off North West coast of India. Different studies in coastal waters of India after the south-west monsoon also show a considerable increase in nutrients as well as nitrates (De Sousen et al., 1996; Mccarthy et al., 1999). The annual average nitrate content of surface waters of north eastern Persian Gulf is reported as 0.12ppm (Ebrahimi, 2002), whereas in the present study the average nitrate level of supply canal and Gwatar Gulf is higher (0.3 ppm). This phenomenon can also be related to the higher nutrient content of water currents from Indian Ocean mixing with surface layers of this region as well as nutrient input from adjacent rivers and mangroves.

Nitrite is the ionized form of nitrous acid (HNO_2). The level of nitrous acid depends on total nitrite, pH, temperature, and ionic strength. Nitrite is a product of nitrification and reduction of nitrate in aquatic environments, or produced in the bacterial oxidization process of ammonia into nitrate (Strilling and Philips, 1990). Therefore nitrite concentration in aquatic environments follows the variations in the contents of nitrate and ammonia. The toxicity of nitrite is principally due to its effects on oxygen transport and tissue damage. Nitrite in blood oxidizes hemoglobin to methemoglobin, which is not capable of transporting oxygen (Tomasso et al., 1979). Methemoglobin in fish can be detected by the color of the blood and gills, which turn brown (known as brown blood disease). The same reaction is thought to occur with hemocyanin of shrimps and other crustaceans. The toxicity of nitrite decreases markedly with increasing salinity (Spotte, 1979). It is postulated that the increased concentration of cations in saline water combines with nitrite ion and prevents its uptake from solution. The toxic levels for nitrite (NO_2-N) for short term exposure lie between 8.6 and 15.5 mg/l for shrimp to 500 to 750 mg/l for mollusks (Pope et al., 1981).

The nitrite content of the drainage canals was higher than Gwatar Gulf in our study which can be related to the turbulence and dilution of effluents in the Gwatar Gulf as well as more favorable conditions for the presence of nitrite in the drainage canals. As a proof the maximum average nitrite concentration in this study was recorded at station 6 of drainage canals (0.023±0.004) mg/l, whereas the highest average ammonia level (0.11±0.017) mg/l was also recorded at this station. Therefore, the reduction process of nitrite ion (NO_2) to ammonia ion (NH_4) had contributed to the higher level of ammonia at this station. There is a sharp increase in the nitrite concentration of station 11 (0.072ppm) at the intersection of the drainage canals and the Gwatar Gulf in late October with a gradual and slow declining trend towards station 15. This can be explained by the more frequent flushing and water exchange at this time which coincided with peak feeding, growth phase and biomass of the shrimp stock resulting in accumulation of considerable amounts of decomposing organic solids, nitrate and ammonia in discharged water. Khodami (2005) reported the average concentration of nitrite in this drainage canal in years 2001 and 2002 as 0.009±0.001 ppm and 0.026±0.002 ppm respectively. Comparison of our results with those given by Khodami shows a higher concentration of nitrite in our study compared to her 2001 study, and a lower rate compared to her 2002 results.

The measured concentrations of nitrate and nitrite in Helleh and Delvar region of Hormozgan province were reported as 0.005±0.001ppm and 0.009±0.001ppm respectively (Omidi, 2003), while those of Tiab in northern and southern sites were recorded as 0.05±0.02ppm and 0.41±0.02ppm (Akbarzadeh, 2003). The ANZECC (1992) recommends that the nitrite concentration in coastal waters should not exceed 1 mg/l. The nitrite concentration in southern Persian Gulf along Kuwait coasts was reported as 0.007ppm and those of Bahrain and Sharjeh coasts 0.01ppm and 0.005ppm respectively (Al.Majed et al., 2000). Moreover the annual average concentration of nitrite in the north eastern area of the

Persian Gulf was reported as 0.011ppm (Ebrahimi, 2003). All of these studies indicate that the nitrite contents of the Persian Gulf and Oman Sea are below the limits recommended by the ACC and Pope et al. (1981) higher than the level recommended by Boyd and Clay (1998), but they are much less than 10ppm which is determined by the IEPO for the agricultural effluents.

Ammonia in water is produced from the metabolism of proteins by aquatic animals, which is excreted across the gills and in urine and feces. It is also a by-product of the decomposition of organic matter by bacteria. In water, ammonia exists in two forms; a highly toxic unionized form (NH_3) and a much less toxic ionized form (NH_4^+). The proportion of ammonia in each form depends on pH, temperature and salinity and normally their molecules are in equilibrium (Boyd and Clay, 1998). In aquatic environments oxygen depletion occurs mainly due to the microbial metabolism of nitrogenous organic matters (Chen, 1992; Boyd and Clay 1998; Boyd and Green, 2002). Ammonia is 'nitrified' to nitrite and then nitrate through a two step process by the 'nitrifying' bacteria *Nitrosomonas* and *Nitrobacter*. Fortunately ammonia is very soluble and is readily used as a nutrient by plants. So when pH rises in ponds with algal blooms, ammonia concentrations tend to be low. According to the ANZECC (1992) the total ammonia nitrogen concentration should not exceed 3mg/l in aquaculture effluents.

As it is evident from figures 10 and 11 the ammonia content of the drainage canals had a gradual increasing trend throughout the culture period especially during September and October. This was mainly because of utilizing urea fertilizers CO_2 (NH_2) over the early rearing stages, accumulation and decomposition of unused feed, shrimps excretions and other organic deposits within the last weeks of the rearing period, along with the frequent flushing and water exchange and final discharge of effluents before harvesting. By comparing the ammonia concentrations in the drainage canals of Gwatar shrimp site in years 2001 (0.075±0.004ppm), 2002 (0.09±0.005ppm) and 2004 (0.086±0.004ppm), we can conclude that in addition to an increase in pH which helps in converting different forms of nitrogen compounds to each other, the total cultured area of the farms had also contributed to the increase in the amount of ammonia. The reported average ammonia concentration in Tiab Shrimp site in 2002 was 1.017±0.011ppm (Akbarzadeh, 2003),

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and its amounts at Helleh shrimp site in years 1998, 2000, 2001 and 2002 were 0.17ppm, 0.197ppm, 0.335ppm and 0.34ppm respectively (Omidi, 2003). The average ammonia concentration in Gwatar shrimp site drainage canal was higher than Tiab site, but less than that of Helleh site. However in the present study the amount of ammonia in the supply canal was 0.05±0.004ppm and its average from August 2001 to May 2003 was recorded as 0.069±0.005ppm which stands within the limits recommended by ANZECC (1992), Boyd and Clay (1998) and Pope et al. (1981). According to Akbarzadeh (2003) the average ammonia content in Tiab shrimp site was 0.006ppm, whereas that of Gwatar site was 10 times more than Tiab. This can be attributed to the existence of mangroves along the supply canal. In rearing ponds the unused food, shrimps excretions and degrading detritus and other organic deposits increased the ammonia content of water which was discharged into the drainage canal and eventually into the Gwatar Gulf. The discharged effluents in the Gwatar Gulf diluted and the average ammonia was reduced to 0.048±0.004ppm. It is worth mentioning that the end of harvesting period in Gwatar site coincides with the end of monsoon and an increase in nutrients. The average ammonia concentration in the shrimp effluents was reported as 0.98ppm in Thailand (Dierberg, 1996), 0.037ppm in Malaysia (Yusoff et al., 2001) 0.36-0.56ppm in Mexico (Cordova et al., 1998), and ranged from 0.04 - 1.11ppm in India (Guhathakurta and Kavrrj, 2001). The 96h LC_{50} for *Penaeus monodon*, N-NH_3 was reported 1.7ppm (Allan et al., 1990) and for *Penaeus chinensis* N-NH_3 was 1.9-2.5ppm (Chen and Lin, 1992). According to Boyd and Clay (1998) the recommended amount of ammonia for the coastal ecosystems receiving the shrimp farms effluents can be up to 3 mg/l. On the other hand the recommended level of ammonia which is determined by the IEPO for the agricultural effluents is 0.01-7.8ppm. Therefore we can conclude that the effluent ammonia content of this research was less than the recommended limit by ANZECC (1992) and Boyd and Clay (1998), as well as being within the recommended range, determined by the Iranian Environment Protection Organization for the agricultural effluents. However the amount of these factors in Gwatar Shrimp site were very close and similar to the other shrimp culture sites along the southern coast of Iran.

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ترکیبات مختلف نیتروژن در سه منطقه زیست محیطی در مجتمع پرورش میگوی گواتر (سیستان و بلوچستان)

شراره خدامی[1] ، گیلان عطاران فریمان[2]، جواد قاسم زاده[2] و محمد صدیق مرتضوی[3]

چکیده

به منظور شناخت وضعیت پساب مزارع پرورش میگوی گواتر در شرق شهرستان چابهار در استان سیستان و بلوچستان، ۱۵ ایستگاه در سه منطقه کانال آبرسان (ورودی آب مزارع)، کانال زهکش اصلی (خروجی زهکشهای فرعی) و خلیج گواتر (محل تخلیه پساب) انتخاب گردید. نمونه‌برداری از آب ،طی یک دوره پرورش در سال ۸۳ (تیر تا آذر) جهت اندازه‌گیری نیتریت، نیترات، آمونیوم انجام پذیرفت. نتایج حاصل از این بررسی نشان دهنده این موضوع است که این سه منطقه از نظر زیست محیطی با هم تفاوت دارند.بطوریکه در نیمه دوم پرورش ترکیبات نیتروژن در کانال زهکش و بعد از مانسون در خلیج گواتر افزایش داشته است. نتایج حاصل از آنالیز واریانس یک طرفه با احتمال ۹۹٪ نشان داده است که بین میانگین فاکتورهای اندازه‌گیری شده در زمانهای نمونه برداری و همچنین بین ایستگاهها اختلاف معنی داری وجود دارد($P<۰/۰۱$). با توجه به اینکه ۲۵٪ از مزارع پرورش میگوی منطقه در دوره مورد بررسی فعال بوده‌اند، ترکیبات نیتروژن پساب مزارع پرورش میگوی گواتر در محدودهٔ مجاز قرار دارد.

واژگان کلیدی: پرورش، مزارع میگو، پساب، آلودگی، مانسون، مواد مغذی، گواتر، اثرات زیست محیطی

۱-موسسه تحقیقات شیلات ایران – صندوق پستی : ۶۱۱۶- ۱۴۱۵۵ – تهران، ایران.

۲-دانشکده علوم دریایی- دانشگاه در یانوردی و علوم دریایی چابهار، ایران.

۳-پژوهشکده اکولوژی خلیج فارس و دریای عمان، بندرعباس صندوق پستی :۱۵۹۷، ایران.

*آدرس پست الکترونیکی نویسنده مسئول:Shkhodami@yahoo.com

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