Heavy Metal Contamination in Indochinese Molluscivorous Catfish (*Helicophagus leptorhynchus* Ng & Kottelat, 2000) from Mun River Basin, Ubon Ratchathani Province

Jarungjit Grudpan¹,² and Kantimanee Phanwichien Pradermwong¹*

**ABSTRACT**

Concentrations of heavy metals (cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), manganese (Mn) and zinc (Zn)) were determined in the liver, kidney, and muscle of *Helicophagus leptorhynchus* found in the Mun River, the longest Mekong tributary in Thailand. Sampling was conducted in three sites, covering the area from up- to downstreams. The concentration of Zn in the fish tissues was significantly higher than those of other heavy metals. Variation in concentrations of heavy metals was observed, and they varied according to space and time. The patterns of metal allocation in the studied organs were (a) liver: Zn > Cu > Cr > Cd > Mn > Pb, (b) kidney: Zn > Cr > Cd > Cu > Mn > Pb and (c) muscle: Zn > Cr > Mn > Cu > Cd > Pb.

**Keywords:** *Helicophagus leptorhynchus*, fish tissues, heavy metals

**INTRODUCTION**

Concern on the effect of anthropogenic pollution to freshwater ecosystems is becoming vital since large amounts of the pollutants are run-off from roads, communities, industrial and agricultural areas worldwide (Langston *et al*., 1999). Among anthropogenic pollutants, heavy metals are of particular concern, due to their potential toxic effect and ability to bioaccumulate, i.e. accumulate in the organism’s tissues and aquatic ecosystems (Censi *et al*., 2006). The distribution of potentially toxic elements in different organs of aquatic living things depends on the element, the species, time of exposure, and exposure route (Marcussen *et al*., 2007). They are, then, eventually accumulated in the food chain and cause ecological damage as well as threaten human health either directly or indirectly.

Risk posed by heavy metals to humans is commonly by means of consumption, in which fish are the major food resource from freshwater ecosystems. Moreover, fish are often at the top of the aquatic food chain and may concentrate large amounts of heavy metals from water (Mansour and Sidky, 2003; Priprem *et al*., 2007). Potentially toxic heavy metals may accumulate in fish because of dietary exposure or absorption through the

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gills (Ruangsomboon and Wongrat, 2006). Thus, commercial and edible species have been widely investigated in order to check for those hazardous to human health (Begüm et al., 2005). Moreover, the metal concentrations in fish can act as an environmental indicator for the integrity or state of the system (Wildianarko et al., 2000). Also, they have the advantage of allowing the comparison of metal concentrations among sites and seasons, where water samples are near or below the detection limit (Ramelow et al., 1989).

Consumption of freshwater fish in the Mekong riparian countries is the highest in the world. The average consumption of freshwater fish per person is approximately 14 kg/person/year, which is six times higher than the world average (Hortle, 2007). Among these fish species, the Pangasiid catfish is one of the dominating fish families being harvested. The Pangasiid catfishes are skin fish, i.e. no scale covers the body and the size of adults varies from less than 40 cm in Pseudolais pleurotaenia to larger than 200 cm in Pangasianodon gigas (Ferraris, 2007). The fishes in this family are also known as migratory riverine fish species that move between upstream refuge and spawning habitats and downstream feeding and nursery habitats. The Pangasiid catfish is omnivorous, feeding on algae, higher plants, zooplankton and insects, while larger specimens also take fruit, crustaceans and fish (Rainboth, 1996). The most common and commercial fish species of this family are Pangasianodon hypophthalmus, Pangasius macronema, Pangasius bocourti and Helicophagus leptorhynchus (Valbo-Jorgnensen et al., 2009; Jiwyam et al., 2010). Among these Pangasiid fishes, H. leptorhynchus is of particular concern in terms of heavy metal contamination since this species is benthic feeding which feed exclusively on mollusks (Poulsen et al., 2004). Generally large amounts of heavy metals could accumulate in sediments, which become their main reservoir in the wetlands (Svobodova et al., 2002). Heavy metals accumulating in sediments can affect the concentration of heavy metals in organisms which dwell in these sediments, especially mollusks (Kim and Kim, 2006).

Diets are the main source of toxic bioaccumulation which could be transferred within the aquatic ecosystem and mostly accumulate highly in the top consumers (Ruangsomboon and Wongrat, 2006). Most of the studies in the contamination of heavy metals in the Mekong countries, so far, are focused either on water, sediment or mollusks. Surprisingly, very few studies have been done relating contamination of heavy metals with freshwater fish in the basin because fish are top consumers in the aquatic food web, though they are among the major protein sources (for example Ruangsomboon and Wongrat, 2006; Marcussen et al., 2007; Priprem et al., 2007). The objective of the study, then, is to evaluate heavy metal concentrations (i.e. Zn, Mn, Cu, Pb, Cr and Cd) in H. leptorhynchus from different organs and compare them in terms of spatio-temporal approach as well as the differences among organs per se.

MATERIALS AND METHODS

Sampling locations and samples

The Mun River is the largest Mekong tributary in Thailand (117,000 km² or 75%
of the Khorat Plateau) and the longest (641 km) in northeastern Thailand. A run-of-the-river hydropower dam called the Pak Mun Dam is located 6 km upstream from the confluence with the Mekong mainstream, in addition to a number of irrigation dams along the river, creating a cascade (Jutagate et al., 2005). Three sites were selected in the lowland portion of the river from the upstream to downstream in Ubon Ratchathani Province, namely (A) Wangyang, Warin Chamrap (15° 10’ 769.0” N 104° 43’ 117.0” E), (B) Buatha, Sawang Weerawong (15° 14’ 30.4” N 104° 57’ 17.9” E), and, (C) Bandan, Khong Chiam (15° 19’ 10.6” N 105° 29’ 47.1” E).

The three sampling sites reflected three separated areas (Fig. 1). The first site represents the most upper reach of the study at the confluences between Mun and Chi Rivers, before the run through the city of Ubon Ratchathani. The second site is downstream of the city of Ubon Ratchathani, and the third site is at the confluence of the Mun River to the Mekong mainstream. Fish were sampled 3 times, representing the 3 seasons (summer, rainy and winter) between October 2009 and September 2010. A total of 397 specimens were collected, i.e. 96 from Wangyang, 173 from Buatha and 128 from Bandan. The size range of the samples was between 13.2 and 44.0 cm SL. Fish samples at very fresh condition were dissected to obtain the targeted tissue separately, i.e. liver, kidney and muscle. These tissues were then kept separately in cleaned plastic zip bags and stored in -80°C freezer, until analysis was carried out in the laboratory.

Laboratory

The tissues were analyzed by a modified procedure from the Association of Official Analytical Chemists (AOAC, 1995). One gram of the tissue, i.e. each for liver,
kidney and muscle, was dried for 8 hours at 60°C. Then, 0.2 g of each dried sample was digested by Nitric acid and Perchloric acid. The digested samples were cooled at room temperature, filtered through Whatmann No. 5 and finally the volume was made to 50 ml with distilled water. The concentration was presented as milligram of heavy metal in one kilogram of sample dry weight (mg kg\(^{-1}\) DW\(^{-1}\)). Analysis of the heavy metals (Zn, Mn, Cu, Pb, Cr and Cd) has been done by using Atomic absorption spectrophotometer (flame technic) model: GBC AVANTA.

**Data analyses**

The obtained values of the heavy metals were statistically analyzed, through firstly, one-way analysis of variance (ANOVA) to compare the differences in (a) concentrations of the heavy metals in the samples, (b) the concentrations of each heavy metal by the effect of sampling sites and organs. Secondly, two-way ANOVA, with sites and seasons as factors, was used for determine the effect to concentrations of each heavy metal. Duncan's multiple range post-hoc tests were applied when ANOVA revealed significant differences. Thirdly, the multivariate analysis of variance (MANOVA) was applied to multivariate heavy metals data to determine the effect of sites, seasons and their combinations. Lastly, the correspondence analysis (CA) was applied to graphically characterize the sites and combinations between sites and seasons in relation to the concentration heavy metal. All statistical analyses were carried out with R software (R Development Core Team, 2012).

**RESULTS**

The average concentrations of the six selected heavy metals (Zn, Mn, Cu, Pb, Cr and Cd), in the samples of *Helicophagus leptorhynchus* from the Mun River, were mostly lower than 20 mg kg\(^{-1}\) DW\(^{-1}\), except for Zn (72.24 ± 3.44 mg kg\(^{-1}\) DW\(^{-1}\)), which was significantly higher (*F*-test, \(F_{5,414} = 201.40, P\text{-value} < 2 \times 10^{-6}\)) than the others (Fig. 2), followed by Cu (16.77 ± 2.50 mg kg\(^{-1}\) DW\(^{-1}\)). The lowest contaminant level was Pb (3.66 ± 0.72 mg kg\(^{-1}\) DW\(^{-1}\)) but it was not significantly different from Mn. It is also worthy to note that extreme concentrations (i.e. outliers) were observed in all heavy metals except Cr.

The multivariate analysis of variance (MANOVA) revealed significant differences in the overall concentration of the heavy metals depending on stations (*Pillai's test, \(F_{2,61} = 7.56, P\text{-value} = 3.92 \times 10^{-10}\)) and its combination to seasons (*Pillai's test, \(F_{4,61} = 1.74, P\text{-value} = 0.019\); Table 1). The correspondence analysis (CA) illustrated the effect of stations (Fig. 3) to each heavy metal. The two axes (first and second CAs) explained 100% and 87.4% of the total inertia in the first and second CAs, respectively. In the first CA, i.e. effected by stations (Fig. 3), Pb dominated in the tissues from Wangyang samples, meanwhile Cd and Cu did for Bandan samples. The short arms of the three remaining heavy metals, on the other hand, implied that they were prevalent in the tissues of *H. leptorhynchus* from all sampling stations. Although prevalent, statistical differences among stations (Fig. 4) were observed in Zn (*F*-test, \(F_{2,67} = 3.18,\)}
Table 1. MANOVA results on the concentrations of the six heavy metals in *Helicophagus leptorhynchus* from Mun River.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>Pilai’s-value</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stations</td>
<td>2</td>
<td>0.887</td>
<td>7.569</td>
<td>3.92 x 10^{-10}</td>
</tr>
<tr>
<td>Seasons</td>
<td>2</td>
<td>0.289</td>
<td>1.606</td>
<td>0.099</td>
</tr>
<tr>
<td>Stations x Seasons</td>
<td>4</td>
<td>0.603</td>
<td>1/744</td>
<td>0.020</td>
</tr>
</tbody>
</table>

Figure 2. Boxplots presenting the distribution of concentration of six heavy metals in *H. leptorhynchus* from the Mun River. The average associated with standard error, in parenthesis, of each heavy metal is presented above the box. Different letters, behind the averages, indicate statistical different at $\alpha = 0.05$. 
Figure 3. Ordination diagram of the correspondence analysis showing the relationship of sampling stations and selected heavy metals.

Figure 4. Boxplots presenting the distribution of concentration of each heavy metal, among the sampling stations, in *H. leptorhynchus* from the Mun River. The average associated with standard error, in parenthesis, of each heavy metal is presented above the box. The different letters, behind the averages, indicate statistical different at $\alpha = 0.05$. 
P-value = 0.027) and Mn (F-test, $F_{2,67} = 3.61$, P-value= 0.033) but not for Cr (F-test, $F_{2,67} = 0.68$, P-value = 0.51). The second CA, i.e. the effect combinations of stations and seasons (Fig. 5), refined the results of the first CA and showed that the domination of Pb in Wangyang samples was only in the winter, meanwhile Cd and Cu dominated in the Bandan samples all year round. Fluctuations in heavy metals used to discriminate the combinations of stations and seasons were confirmed by means of ANOVA (Table 2). Non-statistical significant difference in concentrations of Zn and Mn, among the combination, made the arrow arms of both metals in Fig. 5. However, a significant difference was found in Mn, which could be attributed by the low variation of the contaminant level to the tissue of *H. leptorhynchus*.

The MANOVA results indicated varied significant differences in the overall concentration of the heavy metals among organs (*Pilai’s test*, $F_{2,67} = 1.23$, P-value = $2.26 \times 10^{-16}$). Range of the concentration of each heavy metal in the tissues of the three organs, i.e. kidney, liver and muscle, are shown in Fig. 6. ANOVA showed that the concentration levels of each heavy metal significantly varied organ by organ. Muscles contained relative less heavy metals compared to the other two organs. The significantly higher (P-value< 0.05) concentrations of Zn, Mn and Cu were encountered in the liver. The contamination level of Pb was also high in the liver but significantly different only with muscle (P-value< 0.05) and not kidney (P-value> 0.05). Meanwhile, kidney contained more quantities (P-value< 0.05) of Cd and Cr than the other two selected organs. The sequences, in terms of concentration, of the heavy metal in each selected organ are listed as (a) liver: Zn > Cu > Cr > Cd > Mn > Pb, (b) kidney: Zn > Cr > Cd > Cu > Mn > Pb and (c) muscle: Zn > Cr > Mn > Cu > Cd > Pb.

Table 2. ANOVA results on the concentrations of heavy metal in *Helicophagus leptorhynchus* from Mun River.

<table>
<thead>
<tr>
<th>Station x Season</th>
<th>Heavy metals (mg kg$^{-1}$ DW$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zn</td>
</tr>
<tr>
<td>Wy_summer</td>
<td>54.68±6.71</td>
</tr>
<tr>
<td>Wy_rainy</td>
<td>62.38±5.13</td>
</tr>
<tr>
<td>Wy_winter</td>
<td>64.94±6.04</td>
</tr>
<tr>
<td>Bt_summer</td>
<td>68.06±5.77</td>
</tr>
<tr>
<td>Bt_rainy</td>
<td>70.69±6.63</td>
</tr>
<tr>
<td>Bt_winter</td>
<td>81.30±8.52</td>
</tr>
<tr>
<td>Bd_summer</td>
<td>80.41±11.80</td>
</tr>
<tr>
<td>Bd_rainy</td>
<td>86.40±13.74</td>
</tr>
<tr>
<td>Bd_winter</td>
<td>81.48±18.32</td>
</tr>
</tbody>
</table>

Note: Different letters (a, b and c) in each column indicate statistical different at $\alpha = 0.05$.
(Wy= Wangyang; Bt= Buatha; Bd= Bandan)
Figure 5. Ordination diagram of the correspondence analysis showing the relationship of combinations of stations x seasons and selected heavy metals. 

Note: Wy = Wangyang; Bt= Buatha; Bd = Bandan

Figure 6. Boxplots presenting the distribution of concentration of each heavy metal in fish tissues, in *H. leptorhynchus* from the Mun River. The average associated with standard error, in parenthesis, of each heavy metal is presented above the box. The different letters, behind the averages, indicate statistical different at $\alpha = 0.05$. 
DISCUSSION

The concentrations of all investigated heavy metals contaminating *H. leptorhynchus* varied among sites and seasons, in which Zn showed the highest concentration level. This heavy metal is, therefore, of serious concern because the Zn concentrations in all samples were either double or triple the acceptable value for Zn in edible fish (i.e. 30 mg kg\(^{-1}\) DW\(^{-1}\); FAO, 1983; FDA 2011). This heavy metal is likely coming from rubber plantations, which are now very popular in northeast of Thailand. Zinc oxide is commonly used as a white pigment for preparing the rubber latex (Fierro, 2006), and the residues could be washed away to the river. In addition, in addition to Zn, the findings revealed that the average concentration levels of all the other heavy metals investigated were more than the permissible concentrations for human consumption (FDA, 2011). This is, therefore, alarming as to the high intensity of heavy metal contamination in *H. leptorhynchus* in the Mun River as well as in the Mekong mainstream. The sources of these heavy metals include surface runoff from agricultural lands or urban wastewater. For example, Pb and Cr are used as pesticides, meanwhile Cu and Pb are from construction sites (Fierro, 2006). Similar results on high concentration of heavy metals in other fishes from nearby rivers, i.e. the Pong and Chi Rivers, were also reported (Priprem *et al*., 2007), although not as high as the levels in *H. leptorhynchus* from the Mun River.

In terms of spatial approach, the impacts from terrestrial effluences were obvious. Wangyang site is surrounded by agricultural areas (*personal observation*), therefore, there is a high chance of contamination by Pb which is used for pest control. Meanwhile, high concentrations of Cd and Cu in Bandan are attributed to the mining activities in Lao PDR (U.S. Geological Survey, 2012) as well as accumulation in the downstream site (Qadir and Malik, 2011). Seasonal differences in rainfall and river flow can also influence metal accumulation and bioavailability (Simkiss and Mason, 1983; Qadir and Malik, 2011). High concentrations of heavy metals tend to increase in the rainy season due to terrestrial runoffs, then accumulate in winter while the flow rate is relatively low (Agarwal *et al*., 2007). Moreover, it has also been reported that Zn and Cu would accumulate more in fishes in the summer due to the increase in temperature (Ibrahim and Omar, 2013).

Variations in contamination of heavy metals were also different among fish organs. In general, fish organs show significant variations in metal accumulation, which is related to differences in uptake, absorption, storage, regulation and excretion abilities of fish species (Agarwal *et al*., 2007; Qadir and Malik, 2011). The present results are in agreement with Velcheva (2006), who reported that heavy metals were significantly higher in tissues in fish liver and kidney than in the muscle. Four heavy metals *viz.*, Zn, Mn, Pb and Cu were accumulated in high concentrations in the liver of *H. leptorhynchus* because fish liver is a storage organ and thus it accumulates the highest level of heavy metals (Priprem *et al*., 2007). This is due to the fact that the liver is a vital organ concerned with basic metabolism and acts like a filter that eliminates unwanted
substances, including heavy metals (Figueiredo et al., 2006). The remaining heavy metals, Cr and Cd were high in the kidney. The reason for Cu to be highest in the kidney is because the fish kidney contains a cystine rich copper binding protein, which is thought to have either a detoxifying or storage function, meanwhile Cd is accumulated and form as metalloprotein complexes in kidney (Ashraf, 2005). The lowest heavy metal concentration is in the muscle. This was an expected result and similar as reported elsewhere. It has been pointed out that the muscle is not an active tissue in accumulating heavy metals in fishes (Legorburu et al., 1988).

Differences in metal concentrations in fish can be attributed to the presence of metal contaminants in surface water (Qadir and Malik, 2011) and hence people who use surface water for consumption must be aware of their possible toxic effect. Thus it is recommended that a monitoring program on heavy metals in freshwater fish in Thailand must be established and a mitigation program must be considered a priority concern to be addressed urgently.

**CONCLUSION**

The high concentration of heavy metals in *H. leptorhynchus* is an alarming sign of environmental integrity and human consumption of this fish from the Mun River. Investigation on this matter should be conducted with other fish species as well. A number of studies reported on the variations in contamination levels of heavy metals among fish species (e.g. Legorburu et al., 1988; Begüm et al., 2005; Velcheva 2006; Priprem et al., 2007). The variability of heavy metals in different species depends on feeding habitats, ecological needs, metabolism, age, size, length of the fish and their habitats (Priprem et al., 2007). This is not only impacting the fishes, but this will also eventually impact the ecological integrity of aquatic resources and to the people around the Mun River. This will be harmful and they must be concerned of this.

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**LITERATURE CITED**


Water Quality Control in Tilapia Closed Culture System Using Filter Feeding Freshwater Clam (*Pilsbryoconcha exilis compressa*)

Theeranuch Wedsuwan¹, Wanna Musig² and Yont Musig¹*

**ABSTRACT**

Evaluation of the possibility of using filter feeder freshwater clam, *Pilsbryoconcha exilis compressa*, for the improvement of water quality in tilapia closed culture system was evaluated in a 2 month outdoor tank experiment. Tilapia was cultured in a closed system with and without freshwater clam for two months. Average values of turbidity, chlorophyll *a*, total particulate matter, particulate organic matter, particulate nitrogen and particulate phosphorus in tilapia-freshwater clam treatment were significantly lower (*P* ≤ 0.05) than those in tilapia only treatment. However, there was no significant difference (*P* ≥ 0.05) between the average values of total ammonia nitrogen from the two treatments. Average values of turbidity, chlorophyll *a*, total particulate matter, particulate organic matter, particulate nitrogen, particulate phosphorus and total ammonia nitrogen were 6.0 ± 11.6 NTU, 24.3 ± 15.8 µg/L, 7.9 ± 6.1 mg/L, 6.4 ± 5.1 mg/L, 1.419 ± 1.342 mg/L, 0.087 ± 0.058 mg/L, and 0.473 ± 0.267 mg/L, respectively, in tilapia-freshwater clam treatment. In comparison, tilapia only treatment yielded average values of 22.0 ± 11.6 NTU, 324.1 ± 222.2 µg/L, 42.5 ± 26.2 mg/L, 34.1 ± 21.1 mg/L, 5.291 ± 5.634 mg/L, 0.305 ± 0.189 mg/L and 0.599 ± 0.752 mg/L, respectively. According to the results of this experiment, *P. exilis compressa* is effective in removing particulate matter, particulate nitrogen, phytoplankton and particulate phosphorus from water in tilapia culture tanks resulting in the decrease of 72.7% of turbidity, 81.4% of total particulate matter, 81.2% of particulate organic matter, 92.5% of chlorophyll *a*, 73.2% of particulate nitrogen and 71.5% of particulate phosphorus. There was no significant difference (*P* ≥ 0.05) between production rate, survival rate and growth rate of tilapia in both treatments.

**Keywords:** tilapia, freshwater clam, *Pilsbryoconcha exilis compressa*, closed culture system

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**INTRODUCTION**

In intensive fish culture systems, particulate organic matter consisting of uneaten feed, fish feces, dead and living plankton and other microbes accumulate and become major causes of deterioration of water quality in culture ponds and in water
bodies receiving pond effluents. Total estimated solid wastes output of tilapia was 331-364 kg tonne\(^{-1}\) of feed consumed or 423-496 kg tonne\(^{-1}\) of fish produced. Solid nitrogen waste output was 7.6-8.3 kg tonne\(^{-1}\) of fish produced and solid phosphorus waste output was 5.6-5.9 kg tonne\(^{-1}\) of fish produced. Dissolved N waste output was 40.9-46.2 kg tonne\(^{-1}\) of fish produced and dissolved phosphorus waste output was 4.2-5.0 kg tonne\(^{-1}\) of fish produced (Chowdhury et al., 2013). Part of these solid wastes suspend in water while another part settle down to the bottom. In intensive culture of tilapia, disturbance by fish generate resuspension of bottom sediment. Microalgal growth stimulated by nutrients released from fish waste and uneaten feed also contributes to particulate matter concentration in water in the form of phytoplankton. Because of their potential negative effect, removal of these solids is commonly practiced as a means to manage water quality in water recirculation fish culture systems.

Filter feeding bivalves are very effective in removing suspended solids from water. According to Bayne and Newell (1983) and Bayne and Hawkins (1992), bivalves can clear seston particles greater than 3-µ diameter from water column with high efficiency. Prior to ingestion, filtered particles are sorted, less nutritious and excess particles are immediately rejected as pseudofeces, while ingested material is digested and the remains are excreted as feces. Feces and psuedofeces form biodeposits which settle down to the pond bottom.

Bivalves can utilize particulate organic wastes from aquaculture farms as feed. Excess particulate fish feed released from salmon farms was effectively captured and absorbed by blue mussels, *Mytilus edulis* (MacDonald et al., 2011). Greater increases in shell height and monthly instantaneous growth rates of oysters suspended at a salmon farm were also observed (Jones and Iwama, 1991). Culturing bivalves with fish or shrimp was reported to be able to reduce nutrients and chlorophyll \(a\) concentration in fish ponds (Soto and Mena, 1999; Cordova and Martinez-Porchas, 2006) and reduce nutrient concentration in effluents (Sterling and Okumus, 1995 and Neori et al., 2004 cited by Gifford et al., 2005). Filtration by freshwater bivalves such as the Asiatic clam (*Corbicula fluminia*) and zebra mussel (*Dreissena polymorpha*) reduced water turbidity and phytoplankton concentration and improved water quality in river estuaries and lakes (Phelps, 1994; Leach, 1993).

The freshwater clam, *P. exilis compressa*, is a bivalve commonly found in standing and running waters in Thailand. The clams are collected from natural beds and sold in local markets for human consumption. In this study, we evaluated the possibility of using *P. exilis compressa* to improve water quality of intensive closed culture systems of tilapia by studying the effect of freshwater clam-tilapia co-culture on particulate matter, particulate organic matter particulate nitrogen, particulate phosphorus, chlorophyll \(a\) concentration and other related water quality parameters and production and growth of tilapia.
MATERIALS AND METHODS

The experiment was conducted in a water recirculation culture system without water exchange using 75 x 155 x 60 cm (W x L x H) fiberglass tank. The tank was partitioned into two compartments for tilapia and bivalve by a cement board. The size of the bivalve compartment was 75 x 40 x 60 cm height while the fish compartment was 75 x 115 x 60 cm. In the fish compartment, a 90 cm long baffle was placed perpendicular to the tank partition to direct water flow. The tanks were filled with water at a depth of 50 cm. Water from the lower part of the bivalve section was airlifted into the fish section through two 0.5 inch PVC tubes, flowing around the center baffle back to the bivalve section through a plastic screen on a rectangular hole (15 x 30 cm) on another side of the partition. The hole was cut at 10 cm above tank bottom level (Figure 1). Recirculation rate of water through the airlift system was 4 L/min. Two plastic trays (35 x 55 x 15 cm height) were placed on a PVC stand in the bivalve section. The first tray was placed 10 cm above tank bottom and the second tray was 10 cm above the first tray. Two air stones were placed on the tank bottom under the trays. Stocking rate

Figure 1. Experimental tanks and flow pattern of water between fish compartment and bivalve compartment.
of the bivalve was 3.0 kg/tank (1.5 kg/tray). A preliminary test of the filtering capacity of the clam was conducted to estimate appropriate stocking rate of the clam. Average weight and average number of the bivalves stocked per tank were 18.54 g and 162, respectively. Two air stones were placed in the fish compartment on both sides of the center baffle. Water used in this experiment was stored tap water mixed with water from the tilapia pond. Total volume of water in each experimental tank was 0.58 m³. Tilapia was stocked at the rate of 10 fish/tank. Initial weight of fish was between 70 and 90 g. Water volume in the fish compartment was 0.43 m³ while that in the bivalve compartment was 0.15 m³. Initial stocking rate was 0.76-0.81 kg/0.58 m³ or 1.3-1.4 kg/m³ of total water volume for tilapia and 3.0 kg/0.58 m³ or 5.17 kg/m³ of total water volume for freshwater clam.

The experiment consisted of three replications. Three similar tanks were also set up for control with the same stocking rate of tilapia without bivalve in plastic baskets. Fish were fed twice daily to satiation with 30% protein commercial pelleted feed. Experimental period was 8 weeks. Water samples were collected once a week from the fish compartment to measure dissolved oxygen (DO), pH, turbidity, total particulate matter (TPM), particulate organic matter (POM), particulate nitrogen (PN), particulate phosphorus (PP), chlorophyll a, and total ammonia nitrogen (TAN). Experimental animals were counted and weighed at the beginning and the end of the experiment to calculate survival rate and growth rate.

TPM was analyzed by filtering the samples through GF/C glass fiber filter, dried in an oven at 103-105°C overnight then weighed (APHA et al., 2005). Then the samples were transferred to a muffle furnace and ignited at 450°C for 4 h, cooled in a dessicator and weighed to obtain POM fraction which was equal to the weight lost after 4 h in muffle furnace. PN was analyzed using Kjeltec 1035 analyzer unit, Tecator: Digestion block, Foss, Model 2520. PP was analyzed spectrophotometrically after percholic acid digestion. Chlorophyll a content was analyzed using spectrophotometric determination method after extracting with acetone (APHA et al., 2005). Turbidity was measured by Turbidimeter HACH 2100Q. DO was measured by YSI Dissolved oxygen meter. pH of water was measured by YSI pH meter. TAN was measured by Phenate Method (APHA et al., 2005). Data were analyzed for mean and standard deviation and compared by t-test.

RESULTS AND DISCUSSION

According to the results of this experiment, the introduction of freshwater clam, *P. exilis compressa* into closed culture system of tilapia can improve water quality in the culture system by reducing water turbidity, phytoplankton, total particulate matter, particulate organic matter, particulate nitrogen and particulate phosphorus (Table 1, Figure 2). Average values of turbidity, chlorophyll a, total particulate matter, particulate organic matter, particulate nitrogen and particulate phosphorus in
Table 1. Comparison of average values of turbidity, TPM, POM, chlorophyll a, TAN, PN, PP, DO and pH of water in tanks culturing tilapia and tilapia + freshwater clam (mean±S.D.).

<table>
<thead>
<tr>
<th>Water quality parameter</th>
<th>Initial</th>
<th>Tilapia</th>
<th>tilapia - bivalve</th>
<th>% decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turbidity (NTU)</td>
<td>3.1</td>
<td>22.0±11.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.0±4.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>72.7</td>
</tr>
<tr>
<td>TPM (mg/L)</td>
<td>5.6</td>
<td>42.5±26.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.9±6.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>81.4</td>
</tr>
<tr>
<td>POM (mg/L)</td>
<td>5.1</td>
<td>34.1±21.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.4±5.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>81.2</td>
</tr>
<tr>
<td>Chlorophyll a (µg/L)</td>
<td>11.8</td>
<td>324.1±222.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.3±15.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>92.5</td>
</tr>
<tr>
<td>TAN (mg/L)</td>
<td>0.022</td>
<td>0.599±0.752&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.473±0.267&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>PN (mg/L)</td>
<td>0.199</td>
<td>5.291±5.634&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.419±1.342&lt;sup&gt;b&lt;/sup&gt;</td>
<td>73.2</td>
</tr>
<tr>
<td>PP (mg/L)</td>
<td>0.011</td>
<td>0.305±0.189&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.087±0.058&lt;sup&gt;b&lt;/sup&gt;</td>
<td>71.5</td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td>8.0</td>
<td>7.1±0.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.1±0.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>pH</td>
<td>8.2</td>
<td>8.3±0.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.0±0.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-</td>
</tr>
</tbody>
</table>

Average values denoted by different superscript in each parameter are statistically significant (P≤0.05).

Tanks with tilapia-freshwater clam were significantly lower (P≤0.05) than those in tanks with tilapia only. Average values of turbidity, chlorophyll a, total particulate matter, particulate organic matter, particulate nitrogen and particulate phosphorus in treatment with tilapia and freshwater clam were 6.0 NTU, 24.3 µg/L, 7.9 mg/L, 6.4 mg/L, 1.419 mg/L and 0.087 mg/L, respectively, compared to average values of 22.0 NTU, 324.1 µg/L, 42.5 mg/L, 34.1 mg/L, 5.291 mg/L and 0.305 mg/L of turbidity, chlorophyll a, total particulate matter, particulate organic matter, particulate nitrogen and particulate phosphorus, respectively, in treatment with tilapia only (Table 1). In tilapia only treatment, weekly average values were between 5.2 and 31.0 NTU for turbidity, 42.1 and 658.6 µg/L for chlorophyll a, 8.1 and 75.8 mg/L for total particulate matter, 8.1 and 60.1 mg/L for particulate organic matter, 0.223 and 18.445 mg/L for particulate nitrogen and 0.049 and 0.631 mg/L for particulate phosphorus (Figure 2). Weekly average values of turbidity, chlorophyll a, total particulate matter and particulate organic matter steadily increased from week 1 to week 4. Then average values of turbidity and chlorophyll a continuously decreased from week 5 to week 8 while average values of total particulate matter and particulate organic matter fluctuated a little bit from week 5 to week 6 and then steadily decreased to week 8. Average values of particulate nitrogen increased from week 1 to week 5 then continuously decreased from week 6 to week 8. Average values of particulate phosphorus decreased a little bit from week 1 to week 2 then continuously increased to week 4 before steadily decreased from week 5 to week 8 (Figure 2).

In tilapia-freshwater clam treatment, weekly average values were between 1.4 and 13.2 NTU for turbidity, 7.5 and 53.4 µg/L for chlorophyll a, 1.6 and 19.2 mg/L for total particulate matter, 1.4 and 14.7 mg/L for particulate organic matter, 0.105 and
Figure 2. Average values of total particulate matter, chlorophyll \( a \), turbidity, particulate organic matter, particulate phosphorus and particulate nitrogen in tanks culturing tilapia and tilapia-freshwater clam.
4.167 mg/L for particulate nitrogen and 0.004 and 0.173 mg/L for particulate phosphorus (Figure 2). Average values of these water quality parameters slightly decreased from initial values to the end of the first week. Then average values of these parameters except particulate nitrogen exhibited an increasing trend from week 1 to week 6, then continuously decreased to week 8. Average values of particulate nitrogen increased from week 1 to week 3, dropped down a little bit in week 4, increased again in week 5, and then steadily decreased to week 8 (Figure 2).

The increase of turbidity, total particulate matter, particulate organic matter, particulate nitrogen and particulate phosphorus to maximum values in week 4 and week 5 in tilapia only treatment likely resulted from the growth of phytoplankton which peaked during that period as indicated by the chlorophyll a content. The decrease in turbidity, particulate matter, particulate organic matter, chlorophyll a, particulate nitrogen and particulate phosphorus in both treatments in the last two weeks likely resulted from the heavy rain which caused the overflow of water from the culture tanks during that period.

Results of this study indicated that freshwater clam could reduce turbidity, particulate matter, particulate organic matter, chlorophyll a, particulate nitrogen and particulate phosphorus in water in intensive tilapia closed culture systems by 72.7, 81.4, 81.2, 92.5, 73.2 and 71.5%, respectively. Sterling and Okumus (1995) and Neori, et al. (2004) cited by Gifford, et al. (2005) reported that culturing oyster or mussel with salmon could reduce nutrient concentration in the effluent. Soto and Mena (1999) reported significant decreases of chlorophyll a and total phosphorus in the closed culture system of juvenile salmon with freshwater mussel (Diplodon chilensis) in outdoor tanks compared to controls without bivalves. Chlorophyll a concentration in tanks with mussel was reduced by two orders of magnitude (from ~300 to 3µg/L) compared to tanks without mussels. In addition, total phosphorus was reduced by about one order of magnitude after days 18 to 39. Significantly, the decrease in chlorophyll a was also reported by Cordova and Martinez-Porchas (2006) in earthen pond polyculture of Pacific white shrimp, Litopenaeus vannamei, giant oyster, Crassostrea gigas, and black clam, Chione fluctifraga. The decrease in total particulate matter, particulate organic matter, particulate nitrogen and particulate phosphorus in the tilapia-freshwater clam treatment indicated the possibility of using freshwater clams to improve water quality in intensive tilapia culture systems by reducing particulate organic matter and particulate nutrients from the water column. Filtering suspended particles by bivalves was also reported to have a positive effect concerning aquatic animal disease control. According to Tendencia (2007), the polyculture of green mussels (Perna viridis), brown mussel (Perna Indica), or oyster (Crassostrea sp.) with shrimp could be used to control shrimp disease caused by luminous bacteria.

There was no statistically significant difference (P≥0.05) between overall average values of total ammonia nitrogen in tilapia only treatment and tilapia-freshwater clam
treatment. Average values of total ammonia were 0.599±0.752 mg/L in tilapia only treatment and 0.473±0.267 mg/L in tilapia+freshwater clam treatment (Table 1). Weekly average values (from week 1 to week 8) were between 0.031 and 1.918 mg/L for total ammonia nitrogen in tilapia only treatment and between 0.092 and 0.790 mg/L in tilapia+freshwater clam treatment (Figure 3).

Martinez-Cordova and Martinez-Porchas (2006) reported that polyculture of Pacific white shrimp, *Litopenaeus vannamei*, giant oyster, *Crassostrea gigas*, and black clam, *Chione fluctifraga* in earthen ponds resulted in a significant decrease in total ammonium nitrogen. Soto and Mena (1999) also reported that freshwater mussel, *D. chilensis*, reduced concentration of ammonia about one order of magnitude after from days 18 to 39 in closed culture system of salmon in an outdoor tank experiment. In contrast, the result in this present study indicated that the introduction of freshwater clam (*P. exilis compressa*) into closed culture system of tilapia did not result in the

Figure 3. Average values of total ammonia nitrogen, pH and dissolved oxygen of water in tanks culturing tilapia and tilapia-freshwater clam.
decrease of overall average total ammonia nitrogen concentration in the water. However, in the first two weeks, average values of total ammonia nitrogen in tilapia-freshwater clam treatment were significantly lower (P≤ 0.05) than those in the tilapia only treatment (Figure 3). A high concentration of total ammonia in tilapia only treatment in the first two weeks was likely a result of the combined effects of the accumulation of particulate organic matter which decomposed and released ammonia and the low density of phytoplankton to absorb the ammonia. The average concentration of particulate organic matter at the end of the first week was 8.1 mg/L in tilapia only treatment compared to the average concentration of 1.4 mg/L in tilapia+freshwater clam treatment (Figure 2). The Average concentration chlorophyll a at the end of first week in tilapia only treatment was 42.1 µg/L compared to 3.0 µg/L of average concentration of chlorophyll a in tilapia+freshwater clam treatment. The dense algal bloom in tilapia only treatment beginning from the third week until the end of the experiment in which chlorophyll a content rose to a concentration level of 240.7-658.6 µg/L resulted in steeply decreasing total ammonia nitrogen to the level of 0.031-0.360 mg/L (Figures 2 and 3).

There was no statistically significant difference (P≥0.05) between overall average of dissolved oxygen in tilapia only treatment and tilapia+freshwater clam treatment. Average values of dissolved oxygen was 7.1±0.64 mg/L in tilapia only treatment and 7.1±0.60 mg/L in tilapia+freshwater clam treatment (Table 1). Weekly average values from week 1 to week 8 of dissolved oxygen were between 6.4 and 8.1 mg/L for tilapia only treatment and between 6.4 and 8.0 mg/L in tilapia+freshwater clam treatment (Figure 3).

Overall average of water pH in tilapia only treatment was 8.3±0.30 which was significantly higher (P≤0.05) than the average value of 8.0±0.17 in tilapia+freshwater clam treatment (Table 1). Weekly average values from week 1 to week 8 of water pH were between 7.8 and 8.7 in tilapia only treatment and between 7.7 and 8.2 in tilapia and freshwater clam treatment (Figure 3).

Filtration of particulate matter by freshwater clam removed large quantities of phytoplankton and other particulate matter (uneaten feed, fish feces and dead algal cells) from the water column resulting in the reduction of chlorophyll a, particulate organic matter, particulate nitrogen, particulate phosphorus and turbidity of water. In tilapia-freshwater clam treatment, filtration of particulate matter by the bivalve kept the weekly average total particulate matter and chlorophyll a content less than 19.2 mg/l and 53.4 µg/l, respectively (Figure 2). Large amounts of feces and psuedofeces produced by the freshwater clam were found accumulated at the bottom of the bivalve compartment. In the tilapia only treatment in which no freshwater clam was put into the bivalve baskets, most of the particulate matter also settled down in the bivalve compartment. Bottom sediment disturbance by tilapia generated resuspension of settleable particulate matter which was carried out from the fish compartment with water that flow into the bivalve compartment resulting in small amounts of sediment accumulating in the fish compartments of both treatments.
There were no significant differences in growth performance of fish in both treatments (Table 2). The average total weight at harvest, size at harvest, increase in bodyweight, survival rate and feed conversion ratio of tilapia in the two treatments were not significantly different (P ≥ 0.05). In the tilapia only treatment, average total weight of fish at harvest was 2726.7 ± 64.3 g per tank which was equivalent to a production rate of 4.70 kg/m³. The average body weight of fish at harvest was 272.7 ± 6.4 g. The average increase in body weight was 196.5 ± 7.4 g/fish/8 weeks and the average feed conversion ratio was 1.28 ± 0.04. In the tilapia + freshwater clam treatment, the average total weight of fish at harvest was 2686.7 ± 167.7 g per tank which was equivalent to a production rate of 4.63 kg/m³. The average size of fish at harvest was 268.7 ± 16.8 g. The average increase in body weight was 193.0 ± 20.2 g/fish/8 weeks and the average feed conversion ratio was 1.30 ± 0.06. Survival rate of experimental fish was 100% in both treatments.

The production rate in the tilapia only treatment indicated that at least 4.70 kg/m³ of fish can be produced in closed culture systems without water exchange. However, it has to be noted that part of the culture tank was partitioned and acted as a settling area for settleable particulate matter. Higher production rate of tilapia using this closed culture system may also be expected by co-culture of freshwater bivalve with tilapia using higher stocking rate of fish. However, more investigations are needed in order to obtain proper stocking density of bivalve and fish for optimum production.

Average survival rate of freshwater clam was 65.0 ± 23.3%. High mortality of the clam probably resulted from toxic metabolites generated from the decomposition of large amounts of organic sediment accumulating underneath the bivalve trays. It is likely that this is the main cause of slow growth rate and high mortality of the freshwater clam. The average growth rate of the clam was 0.68 ± 0.24 g/individual/8 weeks. Periodic

Table 2. Survival rate, weight gain, final body weight and feed conversion ratio of tilapia cultured with and without freshwater clam (mean ± S.D.).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tilapia</th>
<th>tilapia - bivalve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial number</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Initial total weight (g)</td>
<td>761.7 ± 53.9</td>
<td>756.7 ± 40.4</td>
</tr>
<tr>
<td>Average initial weight (g/fish)</td>
<td>76.2 ± 5.4</td>
<td>75.7 ± 4.0</td>
</tr>
<tr>
<td>Survival rate (%)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Final total weight (g/tank)</td>
<td>2726.7 ± 64.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2686.7 ± 167.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Final average weight (g/fish)</td>
<td>272.7 ± 6.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>268.7 ± 16.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Average weight gain (g/fish/8 week)</td>
<td>196.5 ± 7.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>193.0 ± 20.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Average weight gain (%)</td>
<td>259.1 ± 25.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>256.4 ± 39.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Feed conversion ratio</td>
<td>1.28 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.30 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Average values denoted by different superscript in each parameter are statistically significant (P ≤ 0.05).
removal of sediments from the bivalve compartment should be an effective means to improve environmental conditions, survival rate and growth rate of the clam. Stocking rate of the clam at the rate of 3 kg per 0.58 m$^3$ of the bivalve compartment may be too high and could also have generated negative effects on the bivalve themselves (Table 3). Proper stocking rate of the clam should improve survival rate and growth of the bivalve. Jones and Iwama (1991) reported greater increases in shell height and monthly instantaneous growth rates of oysters suspended at the salmon farm.

The result of this experiment indicated that freshwater clam-tilapia co-culture can be used as a means to reduce particulate nutrients and particulate organic matter including phytoplankton in intensive culture systems of tilapia even though higher production of fish was not obtained from co-culture of freshwater clam with tilapia at stocking rate using in this study. The reduction of pollutants in culture water resulting from the filtration ability of freshwater clams definitely results in better environmental conditions in culture tanks and less pollutant (organic matter, phytoplankton and nutrients) loading in fish culture effluents.

Table 3. Survival rate, weight gain and final body weight of freshwater clam cultured with tilapia (mean±S.D.).

<table>
<thead>
<tr>
<th>Initial number</th>
<th>162±7.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial total weight (g)</td>
<td>3000</td>
</tr>
<tr>
<td>Average initial weight (g/individual)</td>
<td>18.54±0.78</td>
</tr>
<tr>
<td>Survival rate (%)</td>
<td>65.0±23.3</td>
</tr>
<tr>
<td>Final total weight (g/tank)</td>
<td>2016.7±700.6</td>
</tr>
<tr>
<td>Final average weight (g/clam)</td>
<td>19.22±1.02</td>
</tr>
<tr>
<td>Average weight gain (g/individual/8 weeks)</td>
<td>0.68±0.24</td>
</tr>
<tr>
<td>Average weight gain (%)</td>
<td>3.6±1.2</td>
</tr>
</tbody>
</table>

**CONCLUSION**

According to the results of this study, freshwater clam, *P. exilis compressa*, can be used effectively to improve water quality in intensive tilapia culture systems. Filtration by freshwater clams can significantly reduce total particulate matter, particulate organic matter, phytoplankton, particulate nitrogen and particulate phosphorus in culture water resulting in the reduction of pollutant loading in fish farm effluents and better environmental conditions in fish culture system. However, more studies are needed to obtain proper stocking density of bivalve and fish in order to obtain optimum production as well as farm scale investigation. Finally, hatchery techniques to produce juvenile freshwater clam also need to be developed.

**LITERATURE CITED**


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Contributions

*Kasetsart University Fisheries Research Bulletin* publishes articles on problems and issues in fisheries science and related topics. Acceptable topics include ecosystem and population dynamics, resource assessment, fishing gear technology, fish processing, socio-economics, farming systems, breeding, nutrition, fish health, pollution and aquatic resources management. Articles may be research papers, short communications or invited reviews.

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Only original, unpublished manuscripts not under consideration for publication elsewhere may be submitted. Articles must be technically sound and written in English. The editors will assist authors for whom English is a second language.

Authors must display good knowledge of the primary scientific literature. Authors must also prepare manuscripts according to the journal’s standards and instructions in order to facilitate prompt review and processing of papers.

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1. Organize the research paper logically and clearly, with sections complementing but not repeating each other, as follows:

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   - Materials and methods - includes all crucial information to allow replication of the study
   - Results - gives concise summary of data in Tables and Figures
   - Discussion - places the study in the larger context of fisheries science and literature
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   - References - must substantially include the peer-reviewed primary literature

2. Type the manuscript using Microsoft Office Word software using letter size page (21.5 cm x 28.0 cm) with 2.5 cm margins all around. Double-space the manuscript throughout, including references, Tables and Figure legends. Use Times New Roman font with font size of 10 or 12 points.

3. Leave a triple space before and after all headings. Use capital and lower case letters, never all capitals. Avoid footnotes, addenda or appendices; if they are really important, incorporate them briefly in the text. Underline only the words to be italicized. Define acronyms or unfamiliar abbreviations at first mention in the text. Do not give any acronym in parenthesis if it is not used later again in the text.

4. Give the Latin name and family of the species at first mention in the manuscript. Subsequent references may use the common name. Italicize (or underline) Latin names.


5. Place a (leading) zero before the decimal in numbers less than 1. Give dates in the form 10 January 1994. Spell out numbers less than 10 unless they stand beside standard units of measure (eight fish and 8 kg). Do not spell out numbers larger than 10 unless they are used to start a sentence.

6. Use metric units or the International System of Units (with base units meter, gram, second, liter, mole, joule, etc.). Common units such as day, tons, hectare, watts, horsepower, °C and ppt salinity may be acceptable. Use abbreviations of units only beside numerals (e.g., 5 m); otherwise spell out units (e.g., only meters away). Do not use plural forms or periods for abbreviations of units. Use superscripts and subscripts
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7. In designing Tables and Figures, bear in mind the journal’s page (17.8 cm x 25.3 cm or 7” x 10”) and any reduction needed. Table headings, Figure explanations and other labels must be understandable without reference to the text. Number Tables and Figures consecutively, one per page. Tables must have horizontal lines only at the top and bottom and no vertical lines at all. Leave spaces to indicate groupings of data. Figures must be neat and simple line drawings, computer-generated graphics, or good-quality black and white photographs. Labels or lettering on Figures must be of a size readable after reduction (up to 60%). Send electronic images (.jpg or .tif format) at first submission and the originals only with the revised manuscript if necessary.

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