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DETERMINATION OF LEAD LEVELS IN FARM-RAISED FISH (*OREOCHROMIS SHIRANUS*) IN ZOMBA DISTRICT, MALAŴI

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ABSTRACT

Concentration of lead was measured in muscle tissue of *Oreochromis shiranus* from six artificial ponds in Zomba district, Malaŵi. Samples taken from ponds close to a high-traffic road were compared to a control site which was 1000m away. The mean concentration of lead in muscle tissue of fish samples in this experiment was 0.68 ±0.30mg/kg. Two farms showed significantly greater lead concentrations than the control farm. There was no significant linear relationship ($P>0.05$) between the lead concentration and the distance of the pond from the road. A significant linear relationship ($P<0.05$) and a negative correlation did exist between the level of lead and mass of the fish sample.

Key words: Lead concentrations, *Oreochromis shiranus*, heavy metals.

Introduction

The natural aquatic environment contains a wide variety of elements. Many occur in very low concentrations, like lead, mercury, cadmium and other heavy metals. However, pollution of an aquatic ecosystem can raise the concentration of heavy metals to high levels. This is cause for public health concern, as the consumption of food containing these elements in excess of natural loads can cause serious health problems.

The presence of heavy metals such as lead in our food may be unnoticeable even at toxic levels (Biney *et al*., 1994). Lead contamination is of particular concern, as it is known to inhibit active transport mechanisms involving ATP, to suppress cellular oxidation-reduction reactions and to inhibit protein synthesis (Waldron and Stofen, 1974). This metal also interferes with calcium metabolism and may be a human carcinogen (WHO, 1993). Lead is toxic to both the central and peripheral nervous systems, inducing neurological and behavioural effects. Epidemiological studies indicate that higher blood lead levels are associated with intelligence deficits in children (WHO, 1993).

One of the main sources of lead in the food we eat is contamination in the food chain (Linden, 1995). If an ecosystem becomes contaminated with lead, the organisms present may accumulate lead compounds in their tissues (Adeyeye *et al*., 1996). This bioaccumulation can cause the concentration of heavy metal to be disproportionately high compared to the organism's environment.

The combustion of leaded fuel in automobiles is responsible for the widespread distribution of lead in the world (Biney *et al*., 1994). In southern Africa, where leaded fuel is commonly used, many farms are located near roads that carry high levels of traffic. Products from these farms may show higher levels of lead than those in a more isolated area. Maize, wheat and milk from farms in Kenya showed a higher level of lead when produced close to traffic burning leaded fuel (Kurungu and Tole, 1994). Similarly, fish reared in urban areas can become contaminated with heavy metals that are contributed by automobiles. Lead was detected in the tissues of fish from an urban artificial lake in India (Ruparelia *et al*., 1987), and the consumption of only one fish per day from an urban lake in Cameroon was found to be sufficient to achieve the tolerable intake levels of lead (Demanou and Brummett, 2002). In Nigeria, where the major source of lead pollution is the use of leaded gasoline (Osibanjo and Ajayi, 1980), lead was detected in tissues of *Oreochromis* species from artificial ponds even when the water and soil contained no detectable levels of lead (Adeyeye *et al*., 1996).

In a developing country such as Malaŵi, many people rely on their own small-scale farms to provide food and income. More than one million hectares of land in Malaŵi is cultivated for food, yet about 75% of these holdings are less than 1.5 hectares (ICLARM and GTZ, 1991). The main product is corn (*Zea mays*), but some farmers have integrated small-scale aquaculture into their agricultural practices. The fish produced are a valuable source of dietary protein.

The most widely cultivated fish species in Malaŵi is *Oreochromis shiranus* (Brummett and Noble, 1995), a tilapia that grows well in small ponds. As some of the artificial ponds used for rearing the fish are located near high-traffic roads, there is concern that contaminants from the automobiles may be finding their way into the fish that people eat. Records on the

content of *Oreochromis* species in relation to their distribution seem to be scarce in Malaŵi (Zimba and Kamundi, 2001). These species have been studied in various aquatic environments in Malaŵi for the accumulation of metabolites such as glycogen, protein and fatty acids (Zimba and Kamundi, 2001), and another study assessed the concentrations of some inorganic substances in *O. shiranus* from Bunda Dam (Mumba *et al*., 2001), but lead was not tested. In this study, muscle tissue of farm-raised, adult *O. shiranus* from small-holder farms with integrated aquaculture in Zomba district, Malawi, were tested for concentration of lead.

Materials and Methods

Field Sampling

Fish samples were collected from six small-scale aquaculture farms in Zomba district, Malaŵi, over a period of 5 weeks, from January 31 to March 6, 2003. Five farms were selected based on their proximity to the M1 road that transects the district. These farms are located on both sides of the road, to minimize variations due to prevailing wind direction. A sixth farm, located 1km from the highway, was selected as a control. Five specimens of the species *O. shiranus* were sampled from each site.

Samples were harvested from the pond with a seine net. The specimens were individually measured for weight and length (standard and total), and sexed. Each sample was placed in a self-sealing plastic bag and placed on ice in a cooler box. The samples were stored in a freezer at -15ºC at the National Aquaculture Centre, Domasi until analysis.

At the time of sampling, the water temperature and dissolved oxygen concentration in the fishpond were measured using an OxyGuard Handy Gamma portable dissolved oxygen meter.

Sample preparation for lead determinants

Fish specimens were removed from the freezer and allowed to thaw for one hour. A subsample of about 5.0g of dorsolateral muscle tissue was removed from each fish and weighed on an analytical balance. Each subsample was placed in a 100mL beaker, 10mL of H_2O_2/HNO_3 was added, and the beaker was covered with a watch glass. The digestion reaction was allowed to proceed for about one hour at room temperature, and then the beakers were placed on a hot plate and heated to about 150°C. The solutions were boiled for about one hour, until the volume was reduced to about 5mL. The solution was cooled to room temperature and diluted to 25mL in a volumetric flask. Distilled water blanks and known concentrations of standard lead solutions were also taken through the wet-ashing procedure to test for methodical errors

The sample solutions were transferred to the Geological Surveys Department laboratory in Zomba to be analyzed for lead content using the atomic absorption spectrophotometry (AAS).

Data Analysis

The data was analyzed using the Statistical Package for Social Scientists (SPSS) computer software program. One-way analysis of variance (ANOVA), followed by Dunnett's test for comparing a control mean to each other group mean, was used to identify significant differences between the sample means at α=0.05. Linear regression was performed to determine the relationship between various factors.

Results

The Kamuzu highway in Zomba district has an average traffic frequency of 1080 vehicles per day. The ponds used for fish sampling ranged in size from 83m^2 to 669m^2, with a mean water temperature of 28.2°C and a mean dissolved oxygen concentration of 3.0mg/L. The male to female ratio of the fish samples was 17:13. The mean weight of the fish samples are listed in Table 1.

Table 1: Mean weights and lengths (with standard deviations, ±SD) of fish sampled from farms in Zomba district.

Farm code	Mean weight (g)	Mean length (cm)
Farm A	37 ± 4	10.5 ± 0.6
Farm B	48 ± 14	11.2 ± 1.2
Farm C	36 ± 4	10.0 ± 0.5
Farm D	37 ± 8	10.7 ± 0.5
Farm E	56 ± 15	11.6 ± 1.7
Farm F	83 ± 18	14.5 ± 1.2

The mean level of lead in *O. shiranus* fish muscle by wet weight for each sampling site is shown in Table 2. The distance from the fishpond to the highway is also given.

Table 2: Mean levels of lead (with standard deviations, ±SD) determined in fish muscle for fish sampled in Zomba district, Malawi.

Farm code	Mean level of lead & (±SD) (mg/kg)	Distance of fishpond from the road (m)
Farm A	0.93 ±0.2	8
Farm B	0.75 ±0.4	45
Farm C	0.56 ±0.3	138
Farm D	0.90 ±0.1	41.5
Farm E	0.47 ±0.1	27
Farm F	0.48 ±0.2	1000

The variation in lead levels can be seen clearly in Figure 2, with the control site, Farm F, showing a concentration similar to other farms.

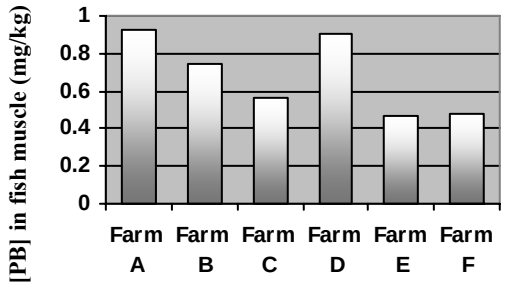

Figure 2: Lead concentrations in muscle tissue of *Oreochromis shiranus* specimens collected from small-scale fish farms in Zomba district, Malawi.

Lead concentration among all the sampled farms was significantly different ($P<0.05$) as revealed by Dunnett's test. Fish sampled from Farm A and Farm D had significantly higher ($P<0.05$) lead concentrations than those from the control site, Farm F.

Linear regression showed that the relationship between the lead concentration and the distance of the fishpond to the road was weak. The Pearson correlation for this relationship was –0.280. The proportion of the total variation accounted for (R^2) by the variation in distance was 7.9%. ANOVA for this model showed that the linear relationship was not significant ($P>0.05$).

Similarly, the relationship between the concentration of lead and the length of the fish samples was weak. The Pearson correlation was –0.311, and 9.7% of the variation was accounted for. The linear relationship was determined to be not significant ($P>0.05$) by ANOVA.

However, linear regression of the variables for the concentration of lead in the fish muscle and the mass of the fish samples showed a stronger relationship. The Pearson correlation between the two was -0.479, and 22.9% of the variation in the lead level could be accounted for (R^2) by mass. ANOVA showed that the linear relationship was significant ($P<0.05$).

Discussion

The mean lead concentrations for all sampling sites are below the limit established by the FAO for the muscle tissue of finfish, which is 2.0 mg/kg (Kalulu *et al*., 1987). However, the Provisional Tolerable Weekly Intake (PTWI) of lead as adopted by most countries is 25µg/kg body weight (WHO, 1993). The mean concentration of lead in the muscle tissue of the fish sampled in this experiment was 0.68 ±0.30. At this level, the consumption of 3 fish per week by an average person (70kg) could cause the PTWI to be reached.

The value found in this study is at the higher end of the range of the mean concentration of lead in freshwater fish muscle determined in previous studies in Africa. For the continent of Africa, research has shown lead levels of 0.02 – 0.67 mg/kg fresh weight. The level is lower for countries in Southern Africa, 0.02 – 0.17 mg/kg fresh weight (Biney *et al*., 1994).

The analysis showed that increasing distance from road to pond and the mean lead concentration in the fish tissue did not exhibit a strong linear relationship. This may indicate that nearness to a high-traffic road is not a significant factor in lead contamination for farm-raised fish. A scatterplot of these two factors, with the distance in a logarithmic scale, seems to indicate that some relationship does exist between the two (Figure 3). Further investigations were performed, including curve estimation regression models for logarithmic and exponential decay curves, to evaluate the nature of the relationship. None of the models were found to apply in a significant manner.

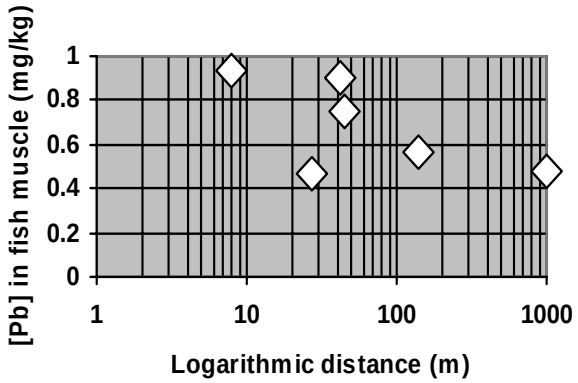

Figure 3: Logarithmic plot of lead concentration (Pb mg/ kg) and distance of fishpond from road.

The scale of this study, however, likely precludes the clarity of this relationship. Investigations into trace metal concentrations call for a high level of precision and even minute variations or contaminations can cause the results to be highly erratic. A larger study would help to eliminate these variations by including duplicate samples for testing, a larger number of samples from each site for more accurate means, and more sampling sites to give a clearer picture of the relationships between various factors.

Such a study would also give more accurate estimations of the means, and allow appropriate measures to be taken to ensure food safety.

Fish tend to bioaccumulate heavy metals in their tissues over time. An older, larger fish might be expected to have a higher concentration of lead in its tissues than a younger fish in the same conditions. While the level of lead in the fish tested in this experiment did not correspond to the length, it did correspond to the mass of the fish. However, the linear relationship was negative, showing that the smaller fish tended to have higher lead levels.

This relationship could be due to a variety of reasons. It is not clear what the route of pollution of lead into the fish tissue may be. Some species of fish consume phytoplankton as part of their diet. These microscopic organisms may absorb heavy metals from the pond water, and therefore the concentration of lead in the water would be a vital factor. It is also possible that lead is being absorbed from the air by crops. Some parts of these crops, such as maize bran, are added to the pond for fish feed. In this case, the accumulation of lead in the fish would be largely dependant on the diet of the fish. A fish being feed increased amounts of contaminated feed would likely exhibit higher lead levels.

If the diet of the fish is poor, there may be a deficiency in certain nutrients or a slower metabolism and in turn less lead accumulation in the tissues.

It is also possible that the presence of lead in the environment and food of a fish in the early stages of its development could result in growth inhibition. Where there are higher levels of lead pollution, a young fry may not develop according to natural processes. This may result in poor metabolism, reduced feed consumption, abnormal growth patterns or reduced body size.

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GROWTH AND EXPLOITATION OF *ENGRAULICYPRIS SARDELLA* IN THE LIGHT ATTRACTION FISHERY OF SOUTHERN LAKE MALAWI.

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ABSTRACT

The growth parameters (L_∞ and K) and mortality coefficients (Z, M and F) for *Engraulicypris sardella* caught from the southern Lake Malawi in the year 2000 using light attraction in Chilimira nets *(*open water seines*)* which are also locally known as called Kauni. fishery were computed using FAO-ICLARM Stock Assessment Tools (FISAT II), Length Frequency Distribution Analysis (LFDA) software and Excel SOLVER routines. Computed growth parameters were used to derive relative yield per recruit (Y'/R) and biomass per recruit (B'/R) which were subsequently used as reference points for exploitation status of the fishery.

The high values of K(0.96, 1.09), L_∞(14.5,13.6) and $-t_0$ (-0.30,-0.055) obtained, were typical of short-lived tropical fishes. High mortality values of Z (2.47, 4.17), M (1.95, 2.15) and F (0.52, 2.02) suggested low annual survival and high turnover rates for *E. sardella.* Recruitment of *E. sardella* was continuous throughout the year with a major peak in July in the southeast arm and with two peaks (in May and September) in the southwest arm stock. Using reference points of E_{max}, $E_{0.1}$ *and* $E_{0.5.}$ current exploitation rates suggest sustainable stocks for *E. sardella* in southern Lake Malawi.

Key words: growth, exploitation, recruitment, *Engraulicypris sardella*

Introduction

Engraulicypris sardella, is an exploited pelagic cyprinid, endemic to Lake Malawi/Niassa, which reaches a maximum length of about 130 mm and it supports a substantial fishery using open water and beach seines (FAO, 1993).

The species is a small shoaling zooplankton feeder in the pelagic ecosystem component of the lake (Turner, 2004). Eccles (1992) reported a maximum total length of 13 cm and that length rarely exceeds 10 cm. Rufli and van Lissa (1982) found that *E. sardella* bred throughout the year but juveniles hatched in the rainy season (November to February) grew faster than ones hatched during the dry season (June and July). The growth rate was more than 0.70 mm TL per day during the rainy season but less than 0.50 mm TL during the dry season.

E. sardella grows to a maximum length of 120–130 mm and is known to occur throughout the lake from very close to the edge to the centre and down to depths of 200 m. Small larvae, less than 5 mm in length, have also been seen in the centre of the lake (FAO, 1992). The observed breeding periods suggest plural stocks adapted to breeding at different temperature optima (Rufli and van Lissa 1982). Ecologically, it is the chief prey of predators like *Rhamphochromis* which inhabit open waters. *E. sardella* is said to be an annual species whose biology is little understood (Thompson *et al.,* 1996).

FAO (1993) confirmed the critical role of *E. sardella* in the economic life of lakeshore communities for food and bait. *E. sardella* is traditionally caught by light attraction in 'Chilimira' nets accounting for 58.8% of 1976-96 catches, the rest being taken by mosquito nets (28.5%) and kambuzi seines (12.3%) (Bulirani *et al.,* 1999). The annual catch was less than 3000 tons during the late 1970s and the early 1980s and the largest catch of 19,000 tons was recorded in 1996. Thus, catch per unit effort has fluctuated greatly over the years but generally high values were observed in the late 1990s (Bulirani *et al.,* 1999).

The standard techniques for the estimation of growth parameters from a time series of length-frequency distributions are well discussed in many fisheries textbooks (Ricker, 1975; Pitcher and Hart, 1990). The use of modal progression in length-frequency plots of sequentially sampled fish populations is also a well-established method of determining growth rates, although precise ages can

only be determined if the cohort is followed from first hatching (Pauly and Morgan, 1987).

The study estimates growth parameters, mortality and exploitation rates, recruitment pattern, yield and biomass per recruit of *E. sardella* in the light attraction from fishery south western and south eastern arms of Lake Malawi.

Materials and Methods

Length frequency data was collected by Malawi Department of Fisheries enumerators from March to November 2000 at Mwawa/Kela and Msaka beaches in southeast and southwest arms, respectively (Figure 1). All fish samples in the study were collected from traditional fishermen who land their catches at nearby beaches for sale to local and further markets. The fishes were caught using light attraction in *Chilimila* nets (open water seines). A total of 1393 and 5562 *E. sardella* were sampled in southeast arm and southwest arm, respectively. Each landing site was visited and sampled for three consecutive days per month at random using total lengths according to Weyl *et. al*. (2004).

The length data was analysed using Length Frequency Distribution Analysis, LFDA version 5.0, (Kirkwood *et al.,* 2001) and FAO-ICLARM Stock Assessment Tools (FISAT II/FAO version 2002).

Growth of *E. sardella* is subsumed to conform to the von Bertalanffy growth model. Estimation of the growth parameters ($L\infty$, K, t_o) of the von Bertalanffy growth curve was defined by grid boundaries using SLCA (Shepherd's Length Composition Analysis) and the ELEFAN (Electronic Length Frequency Analysis). Pairs of $L\infty$ and K estimates were identified using LFDA developed in 1992 and paired values giving maximum score per defined grid after several iterations was selected which were considered to be maximum observed for the species (Kirkwood *et al.,* 2001).

The parameters obtained by Excel SOLVER were used in least squares minimization. Similarly, sum-of-squares residual estimates (RSS) were employed in estimation of least squares analysis both for linear and non-linear functions. Estimates of the least-squares require model parameter values that minimise squared residuals. In turn, assumptions that *E. sardella* of 0.1 year old were 1 cm length in order to fit von Bertalanffy curve from its origin. The growth parameters $L\infty$ and K were used in fitting the von Bertalanffy growth equation:

$$L(t) = L\infty\left[1 - \exp\left(-K\left(t - t_0\right)\right)\right]$$

Where $L(t)$ is the length of the fish at time t; t_0 is the "age" of the fish when L is equal to zero, $L\infty$ is asymptotic length and K is the rate at which the L_t approaches $L\infty$ (Munro, 1982). The growth performance

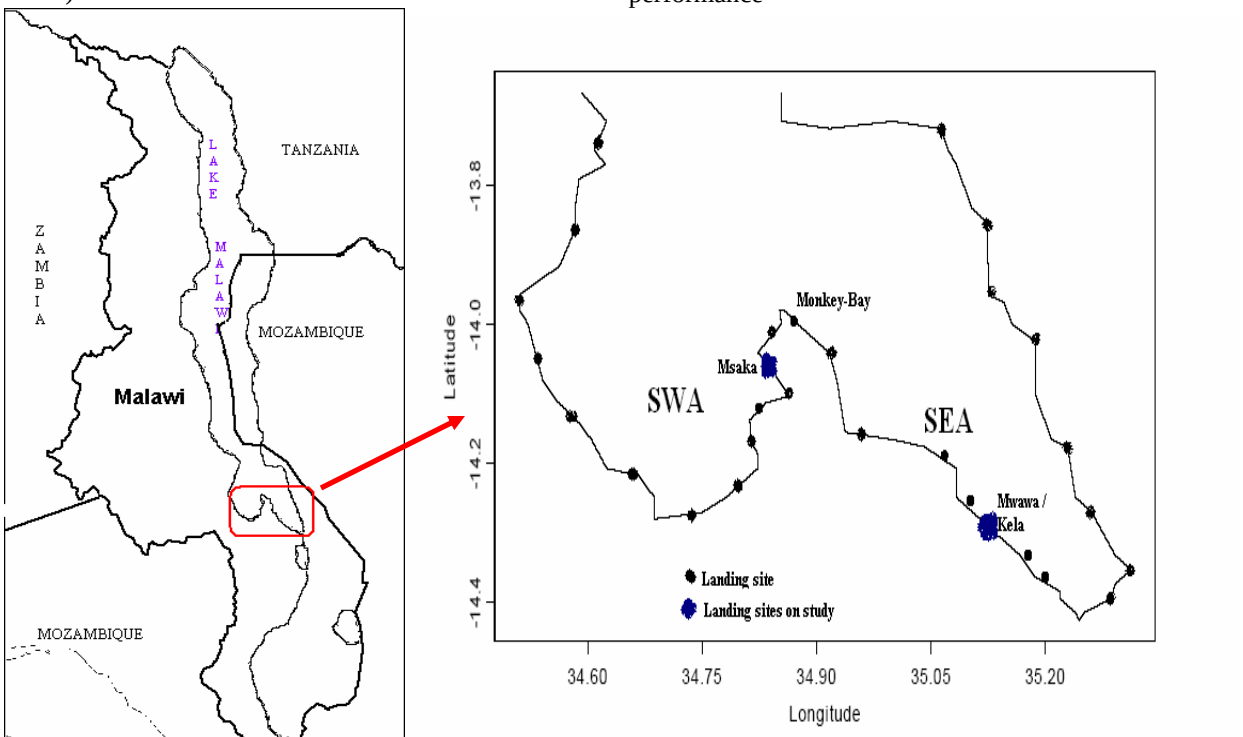


Figure. 1. Map of Malawi showing the study areas Mwawa/Kela in the southeastern arm (SEA), and Msaka in the southwestern arm (SWA).

index (ø'-phi prime) was used to compare overall growth performance of the fish species. The growth performance index, $\phi' = \ln K + 2 \ln L\infty$ (Munro and Pauly 1983) was computed using respective $L\infty$ and K values.

Total mortality coefficient, Z (year^{-1}), was computed using a length-converted catch curve method incorporated in FISAT II from the final estimates of $L\infty$ and K and length distribution data (Gayanilo and Pauly 1997).Assuming constant recruitment and constant mortality, length converted catch curves take the form:

$$\ln\left(\frac{N_i}{dt_i}\right) = a + Z_i$$

Where N is the number of fish in length class i, dt_i is the time needed for the fish to grow through length class i, t_i is age of the mid-length of length class i, Z, with a changed sign, is total mortality and a is a constant.

The natural mortality rate (M) is difficult to estimate in a fully exploited resources (FAO 1993), thus only 'qualified' guesses are made (Sparre *et al*., 1989). The best guess of natural mortality, M (year^{-1}), was estimated using the empirical equation of Pauly (1980) which regresses M on $L\infty$, K and mean environmental temperature, taken as 23 °C in this study from a range of 20 to 27 °C.

$$\ln M = -0.0152 - 0.279 \ln L_\infty + 0.6543 \ln K + 0.463 \ln T$$

Where T is the average annual temperature at the surface in °C. Thereafter, fishing mortality rate (F) was calculated from Z-M. The exploitation rate, E, was computed by dividing F by Z (F/Z), which expresses the proportion of a given cohort/population that ultimately dies due to fishing under existing exploitation pressure according to Beverton and Holt (1966).

ELEFAN in FISAT II was used to obtain seasonal changes in recruitment patterns, and the results are displayed in graphical form. The seasonal recruitment pattern of fish was reconstructed using the entire length-frequency data set subdivided into normally distributed recruitment pulses, representing recruitment seasons in a year. Projecting backward along a trajectory as described by the Von Bertalanffy growth formula using restructured length-frequency data on a 1-year time scale (Pauly, 1987). Maximum likelihood method was used and distribution was resolved into Gaussian components using the NORMSEP (normal separation) procedure of Hasselblad and Tomlinson (1971). Recruitment patterns were presented as percentages of recruitment versus time in months. The number of recruitment peaks in each location was determined.

The yield per-recruit (Y/R) and biomass-per-recruit (B/R) analyses were conducted to obtain reference points and determine the exploitation status. The Y/R model assumes steady state in stock dynamics. Thus, the yield is only valid when the fishing pattern has been the same for a long period of time so that all fish are equally vulnerable to capture after recruitment (Sparre *et al*., 1989).

The model of Pauly and Soriano (1986) was used to predict the relative yield per recruit (Y'/R) as follows:

$$Y'/R = EU^{M/K}\left[1 - \left(\frac{3U}{1+m}\right) + \left(\frac{3U^2}{1+2m}\right) + \left(\frac{3U^3}{1+3m}\right)\right]$$

Where, $E = F/Z$ is the current exploitation rate, i.e. the fraction of mortality caused by fishing activity, F is the instantaneous fishing mortality coefficient, $U = 1-(Lc/L\infty)$ is the fraction of growth to be completed by fish after entry into the exploitation phase (Lc is length at first catch from sampled fish and $L\infty$ is the length of the oldest fish in the stock), $m = (1 - E)/ (M/K) = K/Z$.

The relative biomass per recruit (B'/R) was estimated as:

$$B'/R = \frac{\left(Y'/R\right)}{F}$$

Then, E_{max} (exploitation rate producing maximum yield), $E_{0.1}$ (exploitation rate at which there is marginal increase of Y'/R equivalent to one tenth of its virgin stock) and $E_{0.5}$ is the exploitation rate, which reduces the stock to half its virgin biomass. These were computed through the first derivative of Beverton and Holt (1966) function. Yield contours were plotted to assess the impact of changes in exploitation rate and the critical length ratio $L_c/ L\infty$.

Computations of Y'/R and B'/R were conducted by using Pauly and Soriano (1986) equations, as implemented in FISAT II (Gayanilo and Pauly, 1997) and the knife-edge selection for Y'/R and B'/R calculations. The lowest lengths of L_c = 3 and 4 cm at capture were selected for the south-eastern and south-western arm, respectively.

RESULTS

Mean values obtained from LFDA were used in FISAT II for ELEFAN K scanning, giving L_∞ values of 13.77 - 14.67 cm (±1.4) and K values of 0.8-1.5 yr^{-1} for southeast arm and L_∞ of 12.62 - 14.73 cm (±2.1) and K of 0.89-2.6 yr^{-1} for southwest arm (Figure 2).

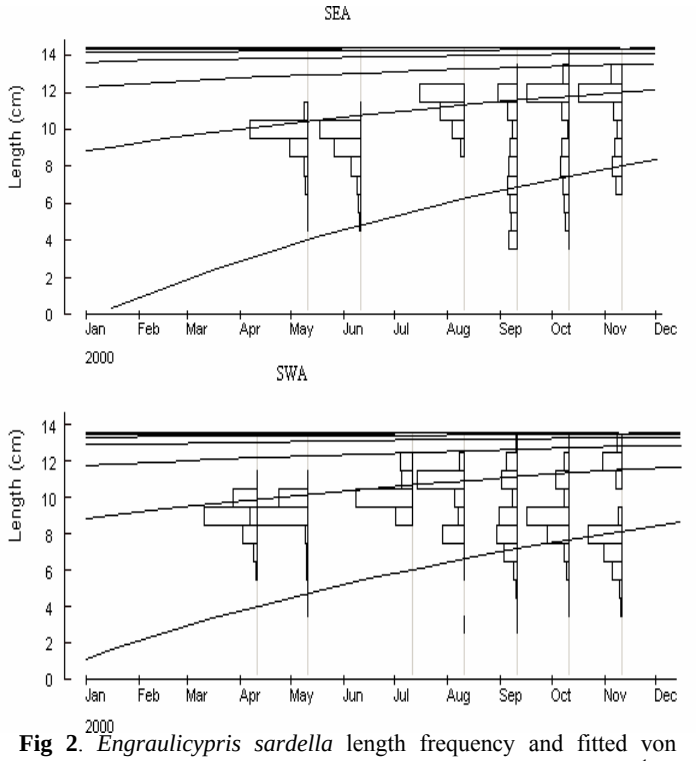
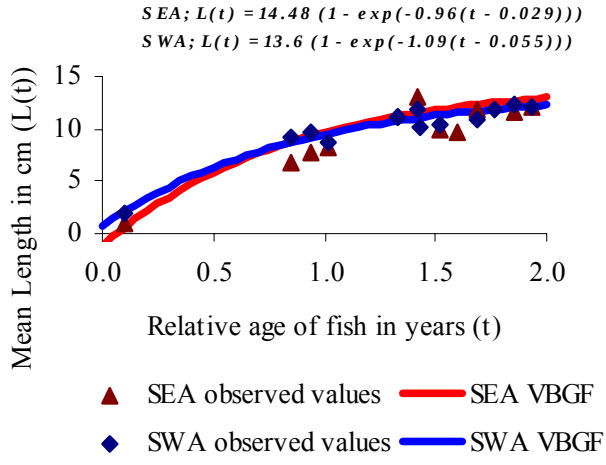


Fig 2. *Engraulicypris sardella* length frequency and fitted von Bertalanff y growth curves ($L\infty$ = 14.5 cm TL, K= 0.96 year $^{-1}$, t_0 = 0) for southeast arm and ($L\infty$ = 13.6 cm TL, K= 1.09 year $^{-1}$, t_0 = 0) for southwest arm

Using minimization routine in Solver (Excel) the von Bertalanffy growth parameter of *E. sardella* for southeast and southwest arm are L_∞ = 14.5 cm and 13.6 cm, respectively, and K = 0.96 yr-1 and 1.09 yr-1, respectively (Figure 3). The Munro's growth performance index (ø) was estimated at 2.3 both locations, an indication of similarity of overall growth performance and conforming to the von Bertalanffy growth (Munro and Pauly, 1983). The L_∞ and K values were within the ranges estimated in FISAT II. However, K values were higher than initial estimates in LFDA.



NB: SEA and SWA mean South East Arm and South West Arm of Lake Malawi respectively

Figure. 3. The von Bertalanffy growth curves for *E. sardella* after sum of square minimisation

Mortality and Exploitation rate

The length converted catch curve analysis for *E. sardella* of the southeast produced lower total mortality estimates of Z = 2.47 yr^{-1} compared to 4.17 yr^{-1} for the southwest for somewhat different relative ages (Figure 4).

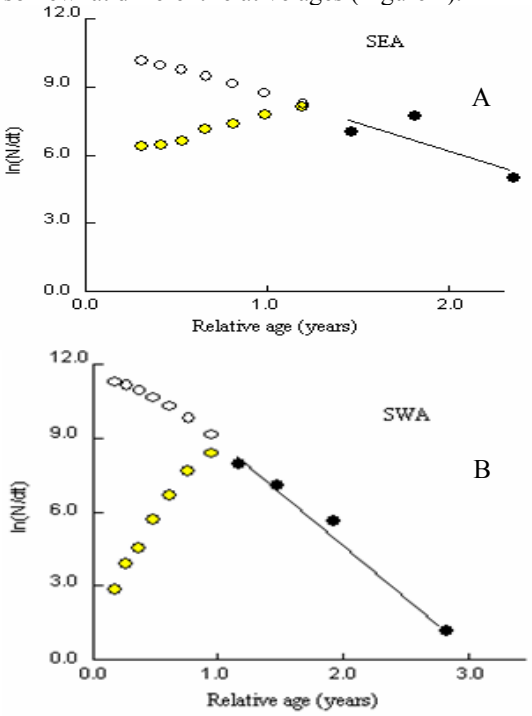


Fig. 4. Length converted catch curves for *E. sardella* caught by light attraction fishery in the southeastern and southwestern arms of Lake Malawi.

A: Length –Converted Catch Curve for Z=2.47; M (at 23.0^{0}c)=1.95; F=0.53; E=0.21

B: Length –Converted Catch Curve for Z=4.17; M (at 23.0^{0}c)=2.15; F=2.02; E=0.48

The natural mortality M = 1.95 for south east arm and 2.15 for south west arm calculated using Pauly's empirical formula (Pauly, 1980) were relatively high for both locations. The fishing mortality F = 2.02 was higher in southwest than in the southeast with F = 0.53. The same was true for the exploitation rate (E = F/Z) as it was higher in southwest E = 0.48 than in the southeast with E = 0.21.

Recruitment pattern

The FISAT II plot of the percentage recruitment of *E. sardella* into the fishery in the portions of the lake showed continuous recruitment from January to November with a peak (≈ 23 %) in July for southeast. Two pulses were recorded in May (≈12 %) and between August to September (≈15 %) in the southwest (Figure 5).

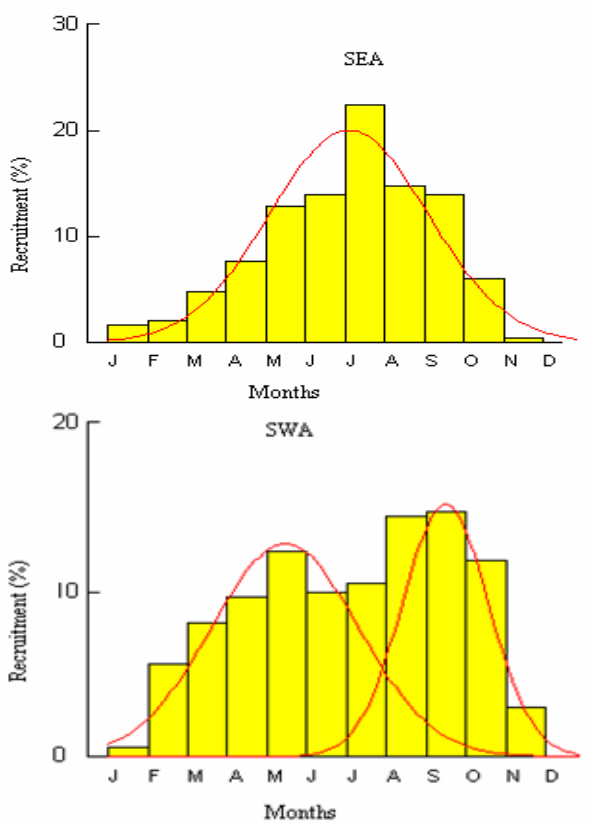
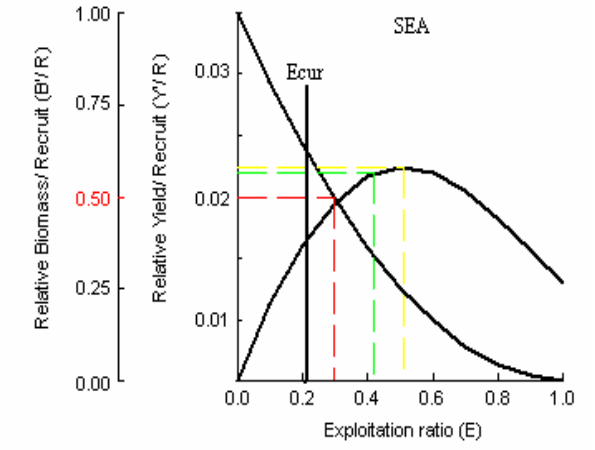


Fig. 5. Recruitment pattern of *E. sardella* caught in the southeast and southwest sections of Lake Malawi (year 2000).

Relative yield and spawning biomass per recruit *(Y'/R, B'/R)*

Using knife edge selection incorporated in FISAT II and assuming constant recruitment the yield per recruit model of *E. sardella* with input parameters *M/K* and *Lc/ L∞,* the optimum exploitation rates were calculated.



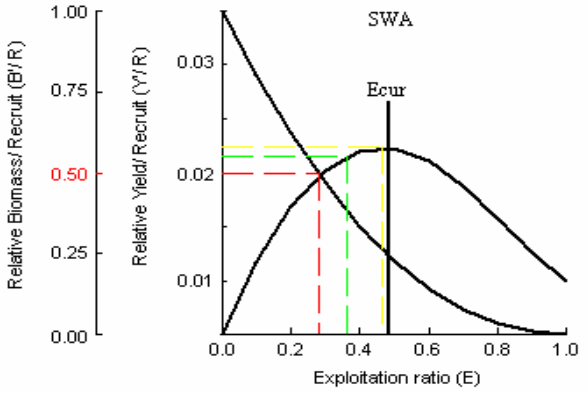


Fig. 6. *E. sardella* Knife-edge relative *Y'/R* and *B'/R* curves in the southeast and southwest Lake Malawi.

The E_{max}, $E_{0.1}$ and $E_{0.5}$ values for relative yield per recruit analysis were 0.51, 0.42 and 0.30 respectively, for the southeast and 0.46, 0.36 and 0.28, respectively, for southwest (Figure 6). The exploitation rate (*E*) for the fishery was 0.21 and 0.45 in southeast and southwest, respectively. Exploitation rate in the southeast was below maximum acceptable limits (*Emax* = 0.51) and the biological optimum (*E0.1* = 0.42). In the southwest, exploitation rate was slightly lower than the maximum acceptable limit (*Emax* = 0.46) but was higher than the biological optimum (*E0.1* =0.36).

Discussion

The growth parameters of *E. sardella* generally agree with previous values reported by Rufli & Van Lissa (1982) and Menz (1995). The von Bertallanffy growth parameter $L\infty$ was close to maximum fish length if we regard the longest sample collected was our L_{max} and t_0 was smaller than zero while *K* varied between zero and one per year, indicating that the fish species is of short life span (Pauly 1978, Moreau *et. al*,. 1986)**.** Previous values of von Bertalanffy for *E. sardella* were L_∞ =108–135 mm and *K* = 0·47–1·64 year^{-1} (Menz, 1995). Rufli & van Lissa (1982) fitted the von Bertalanffy growth curve to stock from Nkhata-Bay (north Lake Malawi) in May-September 1979 and L_∞ = 137 mm and *K* = 2.95 year^{-1}values were reported, which are generally in agreement with results obtained in the current study except for a wider L_∞ range. These results might reflect changes in space and time especially that the current study was on samples obtained in shallow waters, which are also generally biologically more productive.

The growth performance index (*ø'*) was used to evaluate reliability of *L∞* and *K* estimates. Moreau, *et al*. (1986) postulated that environmental conditions like temperature and predation leads to rapid growth towards small size (high *K*, low *L∞*), or slow growth toward large size (low *K*, high *L∞*). The growth indices

of 2.30 and 2.31 were generally in conformity with the pattern reported by Moreau *et al*. (1986).

Recruitment of *E. sardella* was continuous throughout the year with a major peak in July in the southeast but two peaks in May and September in the southwest stock. That *E. sardella* spawns throughout the year was also observed by Morioka and Kaunda (2004). Peak spawning occurred during the rainy season and most juveniles are recruited into the fishery around May. In the southwest, the stronger pulse occurred during February and the second one in August.

Length converted catch curves suggested high mortality (*Z*) values. The exploitation rates of *E. sardella* (E = 0.21 for the southeast and 0.48 for the southwest) are below 0.50. Since $E < E_{opt}$, the stock was considered to be in healthy state. The yield per recruit is maximized at low *E* values only in large, long lived species (Silvestre *et. al*, 2004). For small tropical fishes, high values of *M*, *F* or *E* maximize yield per recruit (Silvestre *et. al*, 2004). Managing a tropical fishery based only on *Y/R* analysis however, could be misleading that is why Pauly and Soriano (1986) and Silvestre *et al.* (1991) cautions about bias that is inherent in assumptions of the knife-edge recruitment model. The natural mortality of *E. sardella* (1.95 and 2.15) was high and attributable to the pelagic behaviour, its relatively short life cycle and feeding on low trophic levels of plankton. This is typical of r-selected fish species (King, 1995). Thus, irrespective of high fishing pressure, biomass fluctuations are expected to be high due to natural mortality, prey-predatory relations (not computed) and other environmental influences.

Gulland (1983) and Pauly (1984) indicated that in small tropical fishes, natural mortality and exploitation rates approach maximum sustainable yield (E_{msy}) and may be unrealistic. Thus they recommend that fishing mortality (*F*) should be low equivalent to the slope of the yield-per-recruit curve of one tenth of the value at the origin of the curve. Current exploitation rate ($E_{current}$) for *E. sardella* exceeds the $E_{0.1}$ value for southwest stocks [E *(0.48)* > $E_{0.1}$ (*0.36*)]. The exploitation rate ($E_{current}$) of *E. sardella* in southeast arm was below $E_{0.1}$ [E (*0.21*) < $E_{0.1}$ (*0.42*)], the optimum.

The relative biomass per recruit (*B/R* or *B'/R*) and relative yield per recruit (Y´/R) curves computed in FISAT II indicate a reference point of $E_{0.5}$, which is defined as the value at which *B'/R* is reduced to half its un fished level. This is the biomass that theoretically maximizes surplus production and generates MSY (Gulland 1983;Pauly 1984).

The theoretical $E_{0.5}$ that maximises surplus production using relative biomass per recruit was 0.3 and 0.28 for *E. sardella* in southeast and southwest stocks, respectively. Except for the southeast arm, the value of the other location exceeded the optimum level of B'/R ($E_{0.5}$).

The relative spawning biomass per recruit is healthy in south - east arm [E (*0.21*) < $E_{0.5}$ (*0.30*)] for *E. sardella* but low and probably unsustainable in southwest arm [E (*0.48*) > $E_{0.5}$ (*0.28*)], an indication of overexploitation. It should also be noted that *Y/R* and *B/R* might be equated to actual yield only where recruitment is constant or varies randomly. Recruitment is unlikely to remain constant at very high *F* or *E* values in the long-term (Pauly, 1984)**.** Therefore due to equilibrium assumptions, yield per recruit models are thought to be more reliable (Gayanilo and Pauly 1997, Pauly and Morgan (1987) and values of *F* or *E* needed to produce maximum yield-per-recruit that tend to generate very low yields because E_{max} usually reduces the parental stock to levels of fewer recruits.

Naturally, high fishing mortality decreases the size at first capture resulting into high yields even in cases where yield-per-recruit analyses predict low yields. The transition period may last several years in fish of high longevity, theoretically sustaining exploitation over many years. In short-lived fish, the transition period tends to be shorter. In *E. sardella*, the distinction between short- and long-term effects is unclear as stocks are not at equilibrium. The same indications were evident after comparing corresponding E_{max}, $E_{0.1}$ and $E_{0.5}$ estimates obtained from *Y/R'* and *B/R'* analyses. Further analysis is required with comprehensive data that cover the whole lake and more time.

Management for *E. sardella* stocks should take into account fluctuations due to environmental factors (Allison *et al.*, 1996). On the other hand, the mesh size of *Chilimira* nets used in the light attraction fishery of *E. sardella* also takes non-target stocks such as as *Oreochromis* spp., other cichlids, clarids and cyprinids as bycatch and inadvertely contributes to overexploitation of other species.

In conclusion *E. sardella* was fished at near maximum sustainable yield in 2000, but a recommendation is made to collect information on prey-predator values and environmental contribution over longer-term period to provide more reliable estimates of growth, recruitment and mortality parameters for management.

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INCIDENCES OF *ESCHERICHIA COLI* AND *SALMONELLA SPP*. IN PROCESSED HAKE FROM COMPANIES BASED AT WALVIS BAY, NAMIBIA.

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ABSTRACT

Microbial contamination of Namibian hakes (*Merluccius capensis* and *Merluccius paradoxus*) by *Escherichia coli* and *Salmonella* spp. were investigated using several culture media. The investigation was undertaken as a part of a training program offered to the students of Fisheries and Marine Science at the University of Namibia. The bacteria were selected to observer compliance to European Export and the Codex Alimentarius Commission Regulations.

Fish samples were obtained from twenty one fish processing plants during January, February and June. Analyses were conducted in collaboration with the South African Bureau of Standards laboratory in Walvis Bay. *Salmonella* spp. were isolated using semi-solid Rappaport-Vassiliadis (RV), Selenite-Cysteine selective media, Xylose Lysine Desoxycholate (XLD) and Brilliant Green (BG) agar. Six colonies of *Salmonella* spp. were identified from one company during the first phase of the study but this could not be consistently demonstrated in other media. *Salmonella* spp. were not found during the second phase of sampling. *Escherichia coli* was absent during both sampling phases, indicating that processing companies generally complied with Codex Alimentarius export requirements. Adoption of zero tolerance for *Salmonella spp.* by Namibian government was generally conformed to by fish processing companies which vindicated the HACCP management system established by South African Bureau of Standards. While this management strategy may serve as an example for other developing countries striving for high quality standards for fish products, referral to other laboratories is required to ensure that there is no conflict of interest. This role might be conveniently played by the University of Namibia.

Keywords: Namibian hakes (*Merluccius capensis, Merluccius paradoxus*), Microbiology, *Salmonella spp*. and *Escherichia coli.*

Introduction

Exports of processed fish are a major source foreign exchange for Namibia. The contribution to Gross Domestic Product increased from N$1, 400 million in 1995 to N$2,292 million in 1999 (Msiska *et al*., 2004). This has enhanced the status of the fishery sector into the second best foreign exchange earner for Namibia after mining (MFMR 2002). The contribution to GDP increased from 5.0% in 1991 to 7.1% in 1998: creating an estimated 14,500 jobs (MFMR 2002). With more than 95% fish products destined for export, it is critical for Namibia to ensure compliance to international quality standards especially for high value fish such as hakes, *Merluccius capensis* and *Merluccius paradoxus* that make an important contribution.

Cases of human contamination usually attract bad publicity in the print media of importing countries and damage the reputation of the industry and country. For instance, outbreaks of food-borne illnesses from fish and shellfish were reported in Denmark (12.4%), Finland (11.9%), France (7.4%), Germany (4.2%), Netherlands (5.9%), Spain (4.1%), Sweden (8.4%), England (3.2%), Wales and Scotland (1.9%) concerning fish products from Senegal, Guinea, Kenya, Uganda, Ivory Coast, Morocco, Nigeria and Mauritania (FAO 1998). This has the effect of eroding consumer confidence. Fortunately, Namibian products were absent from the alert list during the period of reporting, thus confidence in the industry has remained high. The industry has only recently been modernized to incorporate onshore processing following the general resource plunder by pirates which occurred prior to Namibian's independence (in 1990) when most of the fishes were processed on board foreign fishing vessels.

The main sources of fish and shellfish poisoning involve *Salmonella spp.*, Diarrhetic shellfish poisoning, *Vibrio parahaemolyticus*, Histamine, *V. cholera*, *Listeria monocytogenes*, *Staphylococcus spp.*, *Bacillus cereus*, Botulism, Scombroid toxin and

Clostridium bifermentans (FAO 1998, Connell 1995). The pathogens, *Escherichia coli* and *Salmonella spp.,* cause much human sufferings and gain entry into fish products during handling and processing, hence the adoption of HACCP system (FAO 1997). In many developing countries, the monitoring conformity function is outsourced to specialist institutions/agencies due to lack of expertise locally (Connell 1995). Even where this is the case, another referral body is useful. The South African Bureau of Standards is the competent authority responsible for quality conformity in Namibia. However, in order to regulate the industry, the Namibian Government enacted minimum standards of zero bacterial count per gram for *E. coli* and *Salmonella spp.;* these are more stringent than those stipulated by the International Standard Organization 6279 (ISO 1993). For comparative purposes and to ensure repeatability of results, the Rapapport-Vassilliadisis is the medium of choice (ISO 7251:1993) for *Salmonella spp*. isolation. *Salmonella spp*. consists of a large group of bacteria with more than 1,700 serotypes or serovars based on antigen characteristics (Lim 1998), hence it is difficult to identify up to species level. According to Koch (1995), S*almonella spp.* are gram negative, facultative anaerobic, non-spore forming, rod-shaped bacteria, and Kriss (1993), reported certain variations in size and morphology with growth. These bacteria do not ferment lactose instead they use citrate as their source of carbon to produce H_2S; distinguishing them from *Nitrobacteria spp.* (Nester *et al.,* 1998). Human infection by *S. typhi* and *S. paratyphi* leads to gastroenteritis or enteric fever, bacteraemia and squeal sound (a long high-pitch sound) (Gross 1975).

The selective enrichment media for S*almonella* are designed to inhibit growth of other organisms like *Vibrio spp.* and *Shigellae spp.* (ISO 7251:1993). The general selective enrichment media of choice for *Salmonella* are Selenite Cysteine and Rappaport–Vassiliadis although Brilliant Green and Xylose Lysine Desoxycholate (XLD) also support growth (ISO Standard 6579:1993).

The *E.scherichia coli* bacterium lives in water and soil but prefers the gastrointestinal tract. Its genetics, physiology and structure are well known (Lim 1998). *E. coli* is a gram negative, rod shaped, facultative anaerobe that utilizes lactose as a source of carbon (Koch 1995, Nester *et al*., 1998). Under normal conditions, *E. coli* is beneficial to humans as it synthesizes nutrients like Vitamin K and prevents the growth of some potentially harmful bacteria through competition for nutrients and oxygen (Lim 1998). However, when physiological predisposing conditions occur, *E. coli* causes diseases by producing a toxin associated with hemolytic-urea syndrome, which is characterized by lyses of erythrocytes that lead to kidney failure (Lim 1998). In the human body, *E. coli* causes gastroenteritis, urinary tract infection and diarrhea in newborn babies (Gross, 1975).

Various methods are used for the identification of *E. coli* but Laurel Sulphate Tryptose broth and EC broth are the media of choice (ISO 7251:1993). Enumeration is achieved by the "Most Probable Number test (MPN)" of Koch (1995), first proposed by Collins (1967) using MPN tables. Confirmation is shown by Indole production in Kovac `s reagent.

Salmonella spp. and *E.coli* were selected for the study because they are potentially harmful to humans on the other hand. On the other hand, this research was conducted to enhance the reputation of fish processing industry which is an important foreign exchange earner for Namibia. These bacteria are notifiable pathogens in export countries for Namibian fish products.

The specific objectives of the study were to determine the presence and levels of *Salmonella spp.* and *Escherichia coli* on processed Namibian hakes (*Merluccius capensi and Merluccius paradoxus)* and to confirm adherence to the International Standards for export of fish products by the Namibian fishing and processing industries.

Materials and methods

Source of samples

Fish samples were obtained from 21fishing companies and analyzed in conjunction with the South Africa Bureau of Standards (SABS) laboratories (Walvis Bay, Namibia). The SABS was contracted by Namibian Government in 1990 according to ISO 9000 and EN 4500 (EC 2003). The fishing companies export two types of hakes (*Merluccius capensis* and *M. paradoxus*) caught in Namibia `s EEZ (Exclusive Economic Zone) as important part of their product line. Exportable fish and other marine products are physically checked for adulteration, muscle quality and texture, icing, flavor and taste.

Identification and enumeration of bacteria were conducted using standard procedures outlined by Sampling techniques ISO 6279 (1993).

Forty one cartons of fish samples were collected from different fish processing plants based in Walvis Bay. Sampling took place in January, February and June 2004. Specimens were be-headed, gutted and

cut into portions as required for export. The samples were packed in cartons and disinfected with an Alco-bacteria disinfectant. During sampling, it is mandatory for operators to wear protective laboratory coats and gloves and were instructed to strictly follow good laboratory practices to avoid cross contamination of products. Prior to analysis, specimens were defrosted for about 18 hours to reactivate bacterial growth. Portions of 25g were transferred to a sterile stomacher and 225ml peptone buffered water and saline solutions were added to each sample (NaCl, 0.85%W/V) (ISO 6579:1993).

Preparation of culture media

A presumptive test was first used for detecting *E. coli*. McCartney bottles of Lauryl Sulphate Tryptone Broth (LTB) were inoculated with a 25 g of a sample diluted with 225ml of Peptone buffer (Kriss, 1993). Each sample was divided into 9 McCartney bottles of LTB and Durham tubes. Three bottles of Double Strength (D/S) and 6 bottles of Single Strength (S/S) (ISO 7251: 1993) were used for determination of the bacterium.

The following number of bottles were prepared for each broth strengths were prepared as follows: 3 x D/S bottles were filled with 10.0ml of diluted sample solution. 3 x S/S bottles were filled with 1.0ml of diluted sample solution. The development of a gas is a presumptive identification of the presence of *E. coli*.

Nine McConkey bottles of LTB solution (D/S and S/S) were constituded by adding 10.0 ml of a sample solution into 3 D/S bottles and 1.0ml into 3 S/S bottles. A one fold dilution was performed and placed in 3 S/S bottles as shown in the table below:

25g of fish was diluted with 225ml of peptone	3 x bottles of LTB D/S x 10ml of a sample
	3 x bottles of LTB S/S x 10ml of a sample
	3 x bottle of LTB S/S x 10ml of 1 dilution of a sample.

Incubation of the bacterium was conducted for 48 hours at 37ºC. A sample of 0.1ml in EC broth that formed gas was further incubated for 48 hours at 37ºC. A loop of the sample was transferred from the bottle into Tryptone broth at the same temperature, to which Kovac's reagent was added. A cherry-red colour indicates the presence of *E. coli*.

A 25g fish sample was homogenized and diluted to 225ml in a pre-enriched medium. The bacterium was allowed to grow at 37ºC for 16 to 20 hours. It was inoculated into McCartney bottles with RV using a loop containing a portion of the sample. A honey jar with 100ml Selenite-Cysteine was filled with 10ml of a sample using a 10ml drop pipette.

The selenite inoculum was incubated at 37ºC for 24 hours while RV was incubated at 44ºC. Samples were inoculated using a loop onto 2 Brilliant Green (BG) agar plates and 2 XLD for total counts.

Preparation of media for testing *E.coli.*

i. LTB (Lauryl Sulfate Tryptose broth)

A portion of 35.6g of LTB was added into 1litre of distilled water. The solution was dispensed into McCartney bottles containing fermentation (Durham) tubes and autoclaved at 121°C for 15minutes (for single strength). A solution of 35.6g x 2 was used for double strength.

ii. Tryptone

30g of tryptone was added to 1litre distilled water and dispensed into McCartney bottles and autoclaved at 121°C for 15minutes.

iii. EC Broth

7g EC broth was added into 1litre of distilled water. This was dispensed into McCartney bottles and autoclaved at 121°C for 15minutes.

Preparation of media for testing *Salmonella* species.

i. Selenite-Cysteine medium

19g of selenite-cysteine was added to 1litre of distilled water plus 4g of sodium selenite. The solution was mixed well to dissolve the selenite completely, thereafter it was poured into honey jars and autoclaved at 121°C for 15minutes.

ii. Rappaport-Vassiliadis (RV)

42.5g of RV was added to 1litre of distilled water, dispensed into McCartney bottles and autoclaved at 121°C for 15minutes.

iii. Brilliant Green agar

52g of BG agar was added into 1litre of distilled water; the solution was heated gently and boiled to completely dissolve in the medium. The solution was cooled to 50ºC, mixed well and poured onto plates.

Some studies have recommended simultaneous culturing on SS (*Shigella-Salmonella* agar) agar, XLD or $XLDT_4$ and Rambach agars to improve detection level (Yuno *et al*., 1995); however availability of agar was a constraint.

Preparation of agar plates for *Salmonella*

i. XLD agar

52g of XLD agar was poured into 1litre distilled water and allowed to stand for 15 minutes. The solution was heated quickly to boiling point and cooled rapidly to 50ºC. It was poured onto plates at the lowest temperature (37ºC) to prevent spoilage.

Results

A total of forty one samples from 21 fish processing plants designated as A1 to T were tested for both *E. coli* and *Salmonella spp*. Fish samples weighing 25g were used for bacterial counts irrespective of the size of hake. The bar chart in Figure 1 shows the weights of samples used for the study from each company.

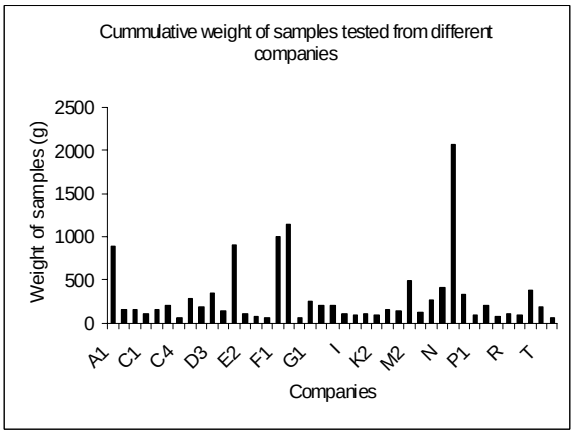

Figure 1. Weights of samples from companies (A-T) used for the study.

Results were generated using the SPSS program: Figures 2-4 were for *E. coli* and Figure 5 was for *Salmonella spp.* Cumulative data are shown in Figure 1 and Figure 2 representing results of 9 x bottles of LTB (for 10 ml D/S, 1 ml S/S and diluted 1 ml of S/S). Figure 3 confirms results of *E. coli* obtained in EC broth and are similar to LTB while Figure 4 was obtained using Tryptone, indicating no response to that medium. Gas formation confirms the presence of the bacterium. The actual concentration of bacteria in the liquid was obtained using MPN tables (ISO 7251).

The LTB solution showed many bottles to have produced gas, especially from companies **D, G** and **I**. Samples from company **M** were the only ones to have 3 bottles forming gas in 1 ml LTB S/S.

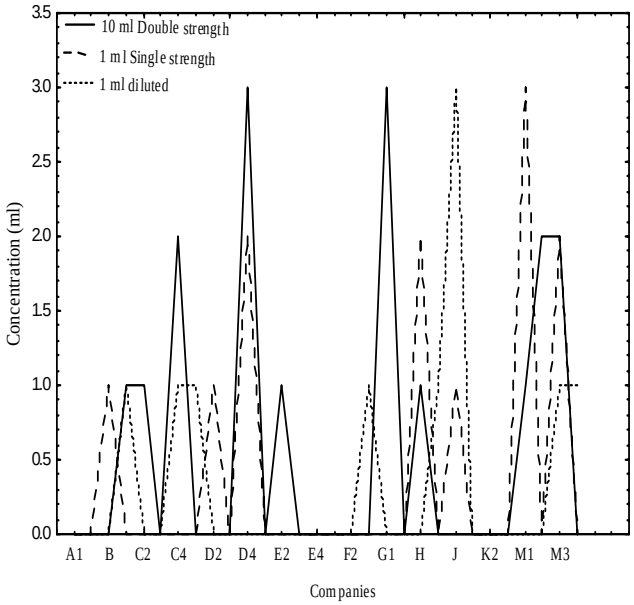

Figure 2. Presumptive identification of *E. coli* in LTB solution

Only company **D** tested positive for 10 ml D/S in EC broth and required further confirmation.

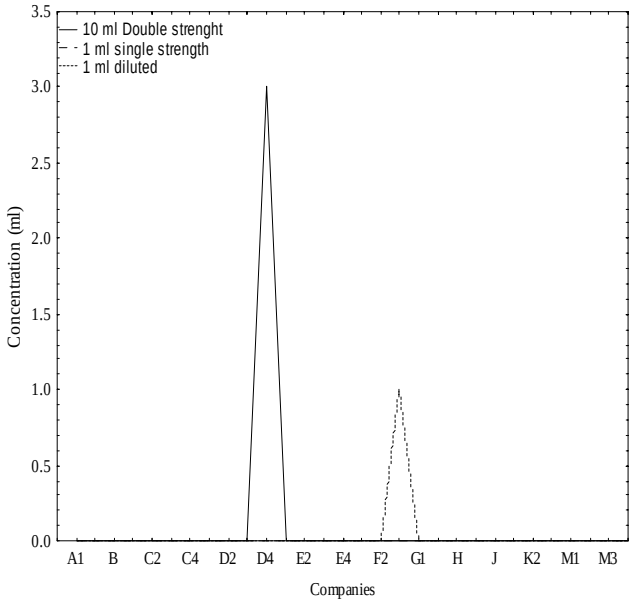

Figure 3. Presumptive identification of *E. coli* in EC broth solution

Bottles that tested positive for *E. coli* in EC broth did not produce gas when further tested using tryptone broth. This meant that there was no *E. coli* in the samples.

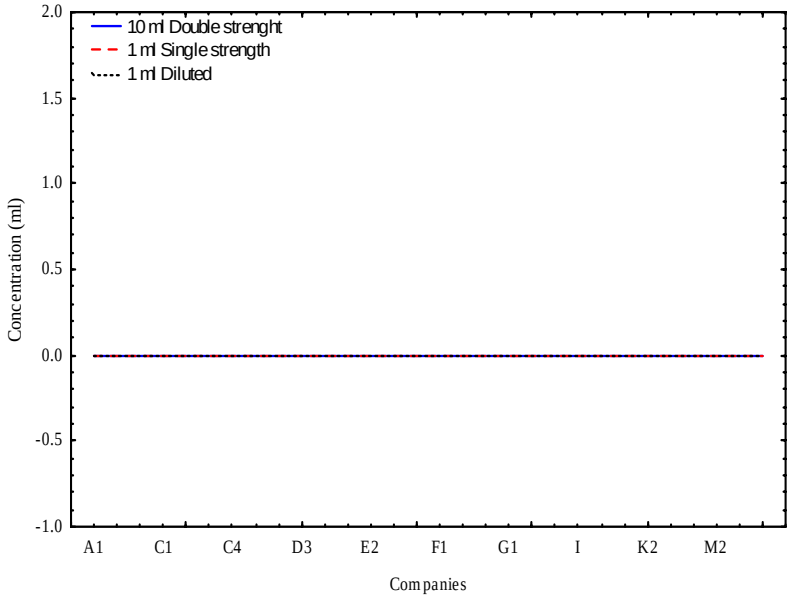

Figure 4. Presumptive identification of *E. coli* in Tryptone solution

The enumeration of S*almonella spp.* using RV and selenite media was successful. The bacterial colonies grown on XLD and BG agar plates were tested. Only a total of 6 bacteria colonies were identified from company **G** during the first sampling. These were further grown on BG and XLD agar plates. On the other hand, there was no *Salmonella spp.* identified from the rest of the companies.

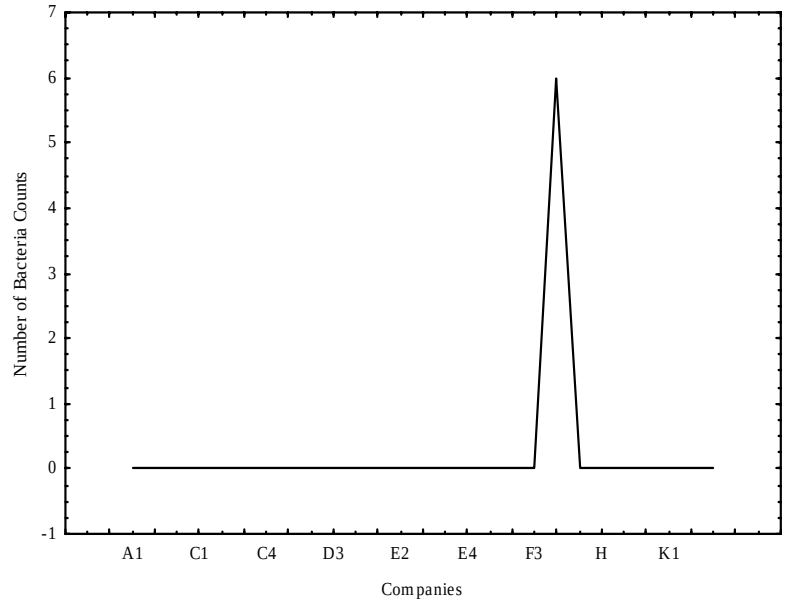

Figure 5. Presumptive identification of *Salmonella spp*. from various companies.

Discussion

Results of presumptive identification using LTB indicated the presence of *E. coli* but further confirmation proved negative. Further propagation in the EC broth resulted only in a few bottles producing gas. According to EU and FAO regulations, processed hake were considered technically free of *E. coli*. Sample sizes were relatively large, hence providing rather large surface area for bacterial growth and serial dilutions confirmed this observation.

With respect to *Salmonella spp.*, it was observed that only company **G** showed positive results during the first phase of sampling. The batch showing positive results was immediately removed from the lot and communicated to inspectors as is the general practice. This illustrates the importance of laboratory testing quickly before exports are made.

The laboratory routines and reporting protocols established suggests that the Namibian fishing industries based in Walvis Bay have efficient services to enforce ISO regulations. This has enhanced the reputation of Namibian fish processed products and has contributed to their promotion in countries of export. But to further consolidate the HACCP management system, there is need for each company to conduct its own laboratory testing to cut on time, otherwise creating a consortium for quality services might be the more economic approach as practiced by Hangana Seafoods (Pty) Ltd which is a merger of several companies.

No *E. coli* but 6 *Salmonella spp.* colonies were found during the first phase of the study (January/ February 2004). These were considered anomalies as they failed to show consistency in the media of choice.

During the last round of sampling (June 2004), virtually no *E. coli* were found and the same was true of *Salmonella spp*. While ISO standards recommend the use of Xylose-Lysine-Desoxyholate medium

for detecting the later, it is also believed that the Xylose-Lysine-Tergitol- 4 medium improves detection level and inhibits *Proteus* (Miller *et al*.,1991). The various forms of *Shigellae spp.* and other intestinal pathogens are also detected in such media, hence the noise registered in samples. Further testing using EC broth is mandatory to provide environmental drag–swabs (Miller *et al*., 1991). These analytical procedures for detecting *Salmonella spp.* could further be improved by using DNA probes, which are faster and more specific (Singleton, 1995).

It is widely accepted that the detection of *E. coli* does not necessarily imply a danger to humans until biochemical marker tests like methylumbelliferyl-beta-glucoronide, sorbitol fermentation, decarboxyllase reactions and hemolysis are conducted to screen the pathogenic forms (Thampuran *et a*l., 2005).

The Namibia Government Gazette (2003) stated that, "there should be zero bacteria count of *Salmonella* and *E. coli* bacteria per grams of exported fish and other marine products." While this regulation is regarded as more stringent than EU and FAO (1997) recommendations, it has to a large extent been attained, even using relatively large sample sizes (25g). Thus the framers of the regulation should be recommended for displaying good foresight. Adoption of the Hazard Analysis Critical Control Point (HACCP) system has generally been good. Thus, the Namibia export sector is justified in demanding premium prices for its marine products. Through consortiums and joint ventures, even smaller companies are able to benefit from premium export prices.

In conclusion, the HACCP practice adopted by Namibian fish processing industries generally ensures safety from food-borne illnesses from fish products. It is therefore not a surprise that the Food Agriculture Organization (FAO) did not place Namibia under alert for notifiable disease contamination through fish products (FAO 1998). Other countries developing export markets for fish and marine products might want to emulate Namibia's HACCP approach. However, since laboratory testing is done only by one company, it is important that there is a referral body, and the University of Namibia could play that role if further capacity is developed within its ranks for this function.

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PHOSPHATE REMOVAL FROM AQUACULTURE EFFLUENT BY ALGAL TURF SCRUBBER TECHNOLOGY

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ABSTRACT

An Algal Turf Scrubber (ATS) was subjected to high effluent loads from African catfish (*Clarius gariepinus*) recirculating aquaculture system (RAS) to examine the effect of total suspended solids load and sludge accumulation in the ATS on phosphate removal. The highest phosphate removal rate (3.42g PO_4-P/m²/d) was higher than values reported from similar research using ATS. Removal of phosphate was more evident in young biofilm than in old biofilm. The ATS ably removed phosphate phosphorus at high rates under Total Suspended Solids (TSS) loading resulting to up to 40g/m²/week sludge dry matter accumulation.

Key words: TSS, phosphate, removal, algae, denitrifying bacteria

Introduction

Effluents with a high concentration of phosphorus (P) and nitrogen (N) are a major problem of the aquaculture industry as they contribute to eutrophication (Smith, 2003). Although Recirculating Aquaculture Systems (RAS) which re-use the water after solid removal and biofiltration considerably reduce the effluent load from aquaculture (Losordo *et al.*, 1998), significant amounts of N and P are still discharged. Sereti *et al.* (2005) successfully replaced with an algal turf scrubber (ATS) the sedimentation unit or both the sedimentation unit and the biofilter in a conventional RAS. The periphyton in the ATS traps organic and mineral matter thereby clarifying the water column. The trapping of particles causes the mats to grow thick and heavy (Verdegem, *et al*, 2005), which influences availability of oxygen (Richmond, 1986). Denitrifying bacteria under anaerobic conditions take up phosphorus in nitrate-rich water. Subsequent increase in oxygen concentration results in release of the phosphorus again by the denitrifying bacteria (Barak and Van Rijn, 2000). Phosphorus can occur as fixed or as soluble phosphorus (SP). SP is the summation of soluble unreactive phosphorus (SUP) and soluble reactive phosphorus (SRP). Orthophosphate, which is taken up by algae is part of SRP. This paper focuses on the removal by an ATS of SRP present in the effluent water of an intensive fish tank loaded with suspended solids.

Materials and methods

The study was conducted in the hatchery "De Haar Vissen" at Wageningen University, The Netherlands. The periphyton based RAS consisted of fish tank, periphyton reactor and a sump (Fig. 1). The ATS was loaded with catfish tank effluent having a flow rate range of 1.11-4.53m³/d.

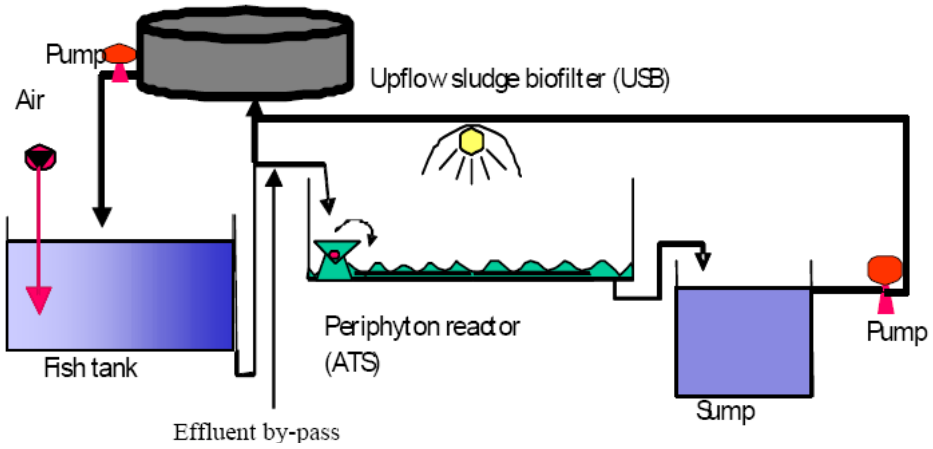

Figure 1: Schematic overview of the experimental set-up

The effluent came from intensive African catfish (*Clarias gariepinus*) rearing tanks.

The ATS consisted of a 2-m² tank with a bottom slope of 5% from inlet to outlet. Two 3-mm mesh stainless steel screens, each 0.9025m² in surface area, were placed in the reactor tank, to support biofilm development. At the inlet of the tank, a tipping plastic bucket was installed, which filled and emptied, creating waves over the screen. The ATS was illuminated by two metal halide lamps (SON-T400W) with 18h:6h L:D photoperiod, providing an average light intensity of 8620 lux.

The catfish tank effluent was the combined flow from 4 tanks. Every 4th week, one of the tanks was harvested and restocked with fingerlings, who were grown in the same tank for 16 weeks. After 16 weeks the tank was harvested. In this way, the feed input fluctuated between a maximum level (the day before tank harvesting) and minimum level (the day of harvest) within each 4 week period (Figure 2).

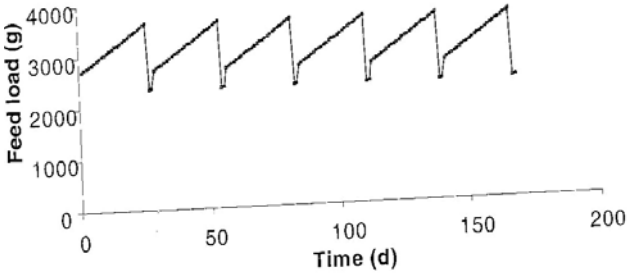

Figure 2: Change in feed loading in the 28 day partial harvest cycles (reflection point) of African catfish (*Clarius gariepinus*) in recirculating aquaculture system

During a 5 week period, the ATS received catfish tank effluents. P removal was evaluated at weekly intervals. Fifty percent of the periphyton on the screens was harvested every seventh day. The hydraulic surface loading (HSL) was determined as:

$$HSL\ (m^3/m^2/day) = Q/S$$

where Q was the flow rate (m³/day) of the catfish tanks effluent and S was the total periphyton screen surface area (m²). Corresponding P loading rates (LR) was calculated as:

$$LR\ (g/m^2/day) = PO_{4in} * HSL,$$

where PO_{4in} is the PO_4 concentration (g/m³) in the catfish tanks effluent at the ATS inlet. The phosphate removal rates (PO_4-P_r) were determined per unit ATS surface area and per unit ATS volume :

$$PO_4\text{-}P_r\ (g/m^2/day) = (PO_{4in}\text{-}PO_{4out})*HSL$$

$$PO_4\text{-}P_r\ (g/m^3/day) = (PO_{4in}\text{-}PO_{4out})*Q/V$$

where PO_{4out} (g/m³) is the phosphate concentration at the reactor outlet,

Where Q is the flow rate (m³/day) and V is the volume (m³) of water in the ATS. Water samples were collected on day 1 and day 7 of each week, simultaneously from both the inlet and outlet of the periphyton reactor. Water samples were collected in 10ml tubes at 1400hrs, for water quality analysis. The 10ml samples were analyzed the same day for phosphate (PO_4-P) using a SAN auto-analyzer (Skalar, The Netherlands). Sludge samples were collected at day 7 in 500-ml bottles. The 500-ml samples were treated with sulphuric acid to lower pH below 2 and were kept under 4°C. They were later analyzed for total suspended solids (TSS) and dry matter (DM) in accordance with APHA (1998). It was assumed that TSS was constant throughout the day and this was used to determine daily TSS loading. Dissolved O_2 (WTW, Oxi 340i, Retsch, The Netherlands), temperature (Testo 110, Retsch, The Netherlands), pH (WTW, pH340, Retsch, The Netherlands) and conductivity (WTW, LF318, Retsch, The Netherlands) were also measured at 14:00hrs, on the first day and seventh day of each week.

Results

Temperature was 25°C during the experiment. pH ranged between 6.83 and 7.72, dissolved oxygen (DO) between 3.88 and 7.36mg/l, whereas conductivity fluctuated between 2.23 and 3.55mS/cm. High DO levels corresponded with higher pH values and were associated with low loading rates.

Sludge dry matter accumulation for each week increased with TSS loading which increased with hydraulic surface loading (HSL) and time from day 1 to day 7 of each week (Fig. 3).

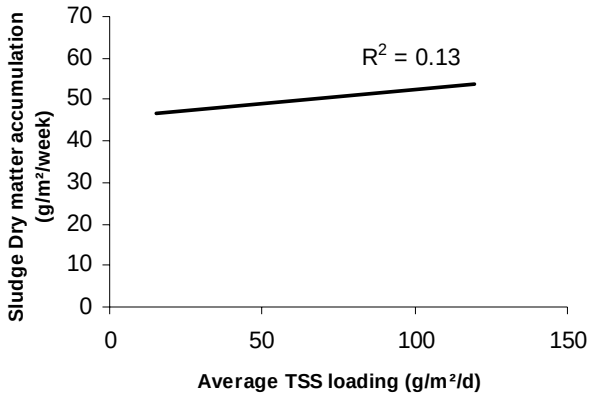

Figure 3: Increase in sludge dry matter accumulation with TSS loading in the ATS

There was more variation in TSS loading (6.2-173.6g/m²/d) at day 1 than (24.8-83.2g/m²/d) at day 7 (Table 1)

Table 1: Total suspended solids (TSS) loading, sludge volume and dry matter accumulation in the ATS at different loading rates of catfish effluent

TSS and sludge load	Week 1	Week 2	Week 3	Week 4	Week 5
TSS loading ($g/m^2/d$) at Day 1	164	6.2	62.4	173.6	26.4
TSS loading ($g/m^2/d$) at Day 7	61.5	24.8	83.2	65.1	26.4
Sludge dry matter(g/week)	45.11	37.31	53.79	58.79	57.23

All the sludge that accumulated under the biofilm plates was taken out on day 7.

Phosphate phosphorus (orthophosphate) removal by biofilm.Net PO_4-P removal in young biofilm increased with TSS loading and dry matter accumulation but removal in a mature (old) biofilm decreased with increased loading (Fig. 4 and 5).

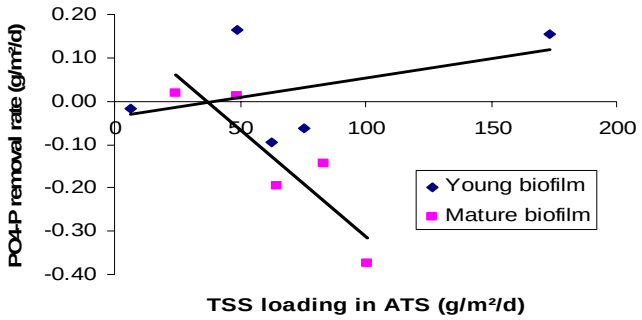

Figure 4: Phosphate phosphorus removal by periphyton in ATS at different TSS loading rates.

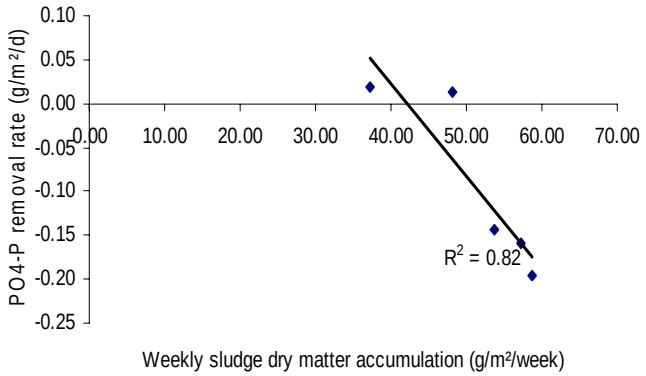

Figure 5: Sludge dry matter accumulation and $P0_4$-P removal by periphyton in an ATS

There was increase in SRP in the ATS effluent beyond 40g/ m^2/week sludge accumulation.

Discussion

The observed removal rates (0.16-3.42 g PO_4-P/m^2/ d) are higher than 0.08 to 0.11g/m^2/d reported by Mulbry and Wilkie (2001). Phosphate (SRP) removal was also higher than 0.032 - 0.036g/m^2/d (Huizing, 2004), and 0.001-0.002g/ m^2/d (Sfetcu, 2003), reported for periphyton reactor and a combination of periphyton reactor and biofilter, respectively. Higher phosphate removal rate was recorded in young biofilm than in mature biofilm. Wetzel, (1993 and 1996), explains that when the biofilm is aged, the dense periphyton community increases the intensity of internal nutrient recycling to the point where the community is maximizing efficiencies of utilization and retention with recycling of the essential resources.

a) Role of algae in the removal of phosphate in an ATS

It is very likely that periphyton (algae) is more responsible for the removal of phosphorus than nitrifying bacteria as indicated by Huizing (2004). Removal of SRP by algae was most likely by filtration, absorption and assimilation and not precipitation as pH was lower than 8.9 (Sfetcu, 2003). Despite the high variation, increased loading of TSS did not have an overall negative impact on PO_4-P removal by algae at day 1 (young biofilm). Partial harvesting of the biofilm allowed for presence of young developing algae which exhibit vigor in taking up orthophosphate for growth (Wetzel, 1993). The newly introduced solids had not yet started to decompose to release more phosphate into the water, while the developing algae maximized uptake of phosphate. Figure 3 shows that accumulation of sludge dry matter somewhat increased with TSS loading (r^2=0.13). It should be noted that there was high TSS loading at high HSL which also implied increased loading of phosphate from the effluent. At high phosphate concentration, more phosphate becomes available for the developing algae. Contrariwise, SRP removal in a mature biofilm decreased with increased loading. Figure 5 shows that SRP removal decreased with increase in dry matter accumulation in the ATS. In fact, beyond 40g/m^2/week sludge accumulation, phosphate released from decomposition of the sludge surpassed the amount removed by the biofilm. At this point the periphyton is aged and has increased in density and thickness, and there is a reduction in the rate of diffusion and penetration of gases and nutrients, hence the decreased uptake of phosphate by the algae.

b) The role of denitrifying bacteria in the removal of phosphate phosphorus in an ATS

Removal by denitrifying bacteria would have been isolated by taking volumetric measurements of nutrient removal from the effluent. However, it is still presumed that denitrifying bacteria played an important role in the removal of phosphate especially in a mature biofilm. During the experiment, gas bubbles were observed forming on top of biofilm as it matured, which was an evidence of nitrogen gas released from the denitrification process coupled with uptake of phosphorus. At day 7, long-term COD (BOD) loads on filter biofilms resulted in decreased nitrification as nitrifiers had less residence time in the aerobic zone, whereas there was higher production of adsorbed organic matter and denitrifying bacteria (Bovendeur, 1989). As anaerobic conditions prevailed denitrifying bacteria took up phosphorus as also observed by Barak and Van Rijn (2000). However, the sum of phosphorus taken up by the denitrifying bacteria and the aged algae was outweighed by the release of phosphate from decaying accumulated sludge in mature biofilm. Thus removal of phosphate in a mature biofilm is not effective.

Uptake and accumulation of soluble phosphorus in an ATS would therefore depend to a great extent on microbial communities present in the effluent, their activity and the time and level of organic matter loading. Future research should aim at quantifying removal of phosphorus by denitrifying bacteria, as compared to algae in an ATS.

Conclusions

A young biofilm is effective in removing phosphate phosphorus in a partially harvested ATS as the developing algae exhibits more vigor. Contrariwise, an old biofilm is not effective in removing phosphorus despite enhanced denitrification due to anaerobic conditions in an ATS.

Acknowledgements

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FLEXIBLE TROPHIC REPERTOIRE: FOOD HABITS OF *RHINOGOBIUS BRUNNEUS* 'ORANGE' (PISCES: *GOBIIDAE*) IN THE ADO RIVER SYSTEM, JAPAN

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ABSTRACT

Trophic dynamics of the potamodromous fish *Rhinogobius brunneus* 'orange' were studied through its food habits from spring to autumn of 1998 in the Ado River system in Japan. The fish was chiefly euryphagous on aquatic insects (Ephemeroptera, Diptera, Trichoptera), attached algae and detritus. Diet changed seasonally and ontogenetically. Feeding intensity was higher in spring and summer than in autumn as indicated by increasing proportion of empty stomachs (summer: 5%, spring: 6%, autumn: 28%) and decreasing index of stomach fullness (spring: 27.3, summer: 24.8, autumn: 13.0). Diet composition diversity increased from spring through summer to autumn. Fish ate more insects in spring (75% of diet) than in autumn (45%) but the opposite was true for algae (spring: 16% of diet, autumn: 44%). Adult fish had a more diverse diet than juveniles in summer and in autumn but juveniles had fuller stomachs in both seasons. Fish had higher condition factors in spring than in summer and in autumn. Periods of high condition factor coincided with those of high feeding intensity and of reported gonadal maturation.

Changing availability and/or suitability of the food resources through the year, size-related differences in foraging microhabitat of the fish and ontogenetic habitat switching by insect prey may explain these diet patterns. Although *R. brunneus* 'orange' is generally euryphagic, it is capable of opportunistically confining or expanding its trophic repertoire. This may be adaptive in the unstable environment in which it lives.

Key words: euryphagous, trophic repertoire, unstable environment, adaptive

Introduction

Studies of resource use, (for example habitat and food) are fundamental in understanding ecological dynamics of community organisation (Begon *et.al.* 1996, Williams 1998, Hill *et al.*, 1999). In fish communities food is one of the most ecologically important resources (Schoener 1974, Ross 1986, Labropoulou and Machias 1998) and studies of feeding ecology may unveil guilds and niche shifts (Hori 1991, Bootsma *et al.*, 1996, Nakano *et al.*, 1999, Maruyama *et al.*, 2001). The coexistence of ecologically similar fish species hinges on their delicate partitioning of this resource (Hyslop 1986, Ross 1986, Moyle *et al*., 1996). Interaction models for ecosystem assessment of fisheries also rely on food consumption estimates (Ngatunga and Allison,. 1996). Dynamics of diet and feeding habits are thus an important aspect of fish biology and ecology (Michelleti and Uieda 1996).

Rhinogobius goby fishes are common in most river systems in Japan (Mizuno *et al.*, 1979), possessing both exclusively freshwater and amphidromous life histories (Mizuno, 1960). They have been extensively studied in Japan (Mizuno 1960, Sunaga 1964, Mizuno *et al.*, 1979, Katoh 1996, Osugi *et al.*, 1998). They are typically benthic in habit, maintaining position on the substrate with their modified pelvic fins when at rest. Adults live in rivers and, after reproduction, their embryos drift downstream into the lake or sea (Iguchi and Mizuno , 1991). After a short pelagic phase, juveniles migrate back into the rivers.

Rhinogobius brunneus 'orange' is one of the three sub-species originally regarded as ecological types of *Rhinogobius similis* Gill (Mizuno 1960). It is now suspected to be a full separate species exclusively associated with the Lake Biwa catchment (Takahashi and Okazaki 2002, Takahashi and Ohara 2004). Although its life history patterns and other aspects of its behaviour have recently attracted some attention (Maruyama *et al.*, 2001; 2003, Okuda *et al.*, 2003; 2004), few sources (Kuwahara *et al.*, 1993, Yuma *et al.*, 2000, Maruyama *et al.*, 2001) have touched on the trophic ecology of the species and none has done so directly. Diet patterns in *Rhinogobius* goby fishes are however, river specific (Kawanabe 1959, Mizuno 1960), and findings

elsewhere may not be as relevant to the Biwa ecosystem. Here we present results of a focused study on the food habits of *Rhinogobius brunneus* 'orange' in the Ado River, one of the largest drainage systems in the Lake Biwa catchment in central Japan.

Materials and methods

All the fish were caught in the Ado river system, a major contributor to Lake Biwa's waters in central Japan (Figure 1).

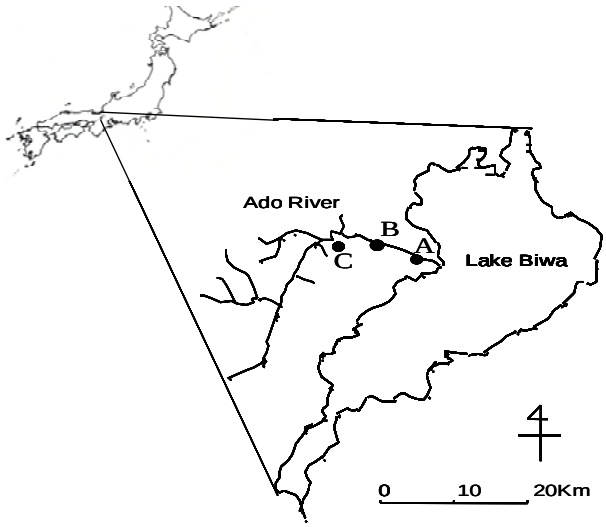


Figure 1. Map showing the location of Ado River in Japan (A, B and C were the sampling points)

Spring samples (n = 37: 3 juveniles and 34 adults) were obtained in May 1998; those for summer in months of June, July and August (n = 118: 11 juveniles and 107 adults) while autumn samples (n = 123: 48 juveniles and 75 adults) were obtained in September and October of the same year. Fish were caught using scooping hand nets (about 2 mm mesh size) on a stretch of about 60m along the river on each site in each sampling month.

Fish were injected in the stomach with and fixed in 10% formalin solution on site. Laboratory analysis included identification of the species using, among other criteria, pectoral fin ray count ($\geq$19 for *R. brunneus* 'orange') and body colour pattern change in formalin (Mizuno 1960), measurements of standard length and wet weight. Based on a minimum of 30mm standard length for maturity (Katoh, 1996) and previous size-class categorisation of the same species (Maruyama *et al.*, 2001) the fish of standard length of up to and including 28 mm were classified as young while those more than 28 mm were considered adult. Since few young fish were obtained in spring only large fish were used in seasonal diet comparisons and for the same reason ontogenetic diet comparisons were done only with summer and autumn data.

Points method (Hynes, 1950) was used in gut content analysis. Each food category was awarded points relative to its estimated contribution to stomach volume under a binocular microscope. Contribution of each food category to a particular fish diet was then estimated by the formula:

$$Pf_{xi} \big/ Pf_i$$

Where Pf_{xi} is the number of points scored for food category x in fish i and Pf_i is the total number of points for all food categories in fish i. The most anterior part of the gut, the loop before the first bend of the alimentary canal, was dissected to reduce difficulties of identification of stomach contents in more posterior sections where digestion was more advanced (Osugi *et al.*, 1998). Insects and algae were identified to the lowest possible taxonomic level. Stomach fullness (an estimate of feeding intensity) was calculated as

$$\left(GCw_i \big/ TBw_i \right) \times 1000$$

Where GCw_i is the gut contents weight of fish i and TBw_i is the total body weight for that fish (Osugi *et al.*, 1998). Shannon-Weaver index was used to calculate diversity of diet composition (Hodson *et al.*, 1981) based on mean proportions of specific food items per size class per season.

$$H' = - \sum_{1}^{s} pi \log_e pi$$

Where s is the number of food categories and *pi* is the proportion of diet occupied by the *i*th category. Pielou's index, $J = H'/ \log_e s$ was used to estimate the evenness of the abundance of food categories in the stomach. $\text{Log}_e s$ is the maximum value of *H'*, which occurs when all food categories eaten are equally abundant (Hodson *et al.*, 1981). Condition factor (K) of the fish was calculated as

$$K = \frac{W \times 100}{L^3}$$

Where W = fish weight (g) and L = fish standard length (cm) (Moyle *et al.*, 1996).

Data analysis

Non-Parametric Multivariate Analysis of variance (NPMANOVA) and the Mann-Whitney U tests were used for statistical assessment of diet differences among seasons and between size classes while Spearman rank correlation tests were used to compare food item dominance between seasons and fish sizes. All these analyses were carried out using statistical package PAST - PAlaeontological STatistics, ver. 1.37 (Hammer *et al.,* 2005).

Results

1. Diet composition and feeding in general

A total of 278 fish stomachs were dissected and examined across the three seasons. Of these 216 were adults (spring n = 34, summer n = 107, autumn n = 75) and 62 (spring n = 3, summer n = 11, autumn n = 48) were juveniles. Over 88% of the 246 stomachs with food items contained insects; about 46% and 35% had algae and detritus, respectively. Thirty three different kinds of items were found in the fish stomachs across the three seasons (spring: n = 32 fish, summer: n =102 fish, autumn: n = 54 fish) (Table 1).

Table 1.Stomach contents of *Rhinogobius brunneus* '(orange' in Ado River, Japan

Order	Family	Genus
Ephemeroptera	Baetiscidae	*Baetisca spp.*
	Behningiidae	*Dolania spp.*
	Ephemerellidae	*Ephemerella spp.*
	Ephemeridae	*Ephemera spp.*
		Hexagenia spp.
Diptera	Ceratopogonidae	*Bezzia spp.*
		Dasyhelea spp.
	Chironomidae	*Ablabesymyia spp.*
		Chironomus spp.
		Pseudosmittia spp.
	Orthocladiinae	*Corynoneuri spp.*
	Stratiomyidae	*Odontomyia spp.*
Trichoptera	Hydropsychidae	*Hydropsyche spp.*
	Others	*Unidentified*
Bacillarriophyta	Achnanthoidae	*Cocconeis spp.*
	Cymbellaceae	*Amphora spp.*
		Cymbella spp.
	Discoidae	*Cyclotella spp.*
		Aulacoseira spp
	Fragilariaceae	*Fragilaria spp.*
		Synedra spp.
	Naviculaceae	*Navicula spp.*
		Stauroneis spp.
	Tabillariaceae	*Tabellaria spp.*
Chlorophyta	Chlorococcales	*Scenedesmus spp.*
	Cladophorophyceae	*Cladophora spp.*
	Desmidiaceace	*Closterium spp.*
		Cosmarum spp.
Cyanophyta	Chroococcaceae	*Chroococcus spp.*
		Merismopedia spp
		Mircocystis spp.
	Oscillatoriaceae	*Oscillatoria spp.*

For further analysis, however, consideration was given to only those food items that made up at least 5% of the overall diet in any one season as a major food item. Based on this, the main food items of this fish were insects of the orders Diptera, Ephemeroptera and Trichoptera, algae (Bacillariophyta, Chlorophyta and Cyanophyta) and detritus.

2. Seasonal variations

a. ***Changes in overall diet composition***

Occurrence of insects in fish stomachs tended to decrease from spring (100%) to autumn (80%) while that of algae and detritus had the opposite trend during the same period. About 38% of fish stomachs had algae in spring but this frequency rose to almost 50% by autumn. Detritus occurrence more than doubled from about 19% of fish stomachs in spring to about 41% by autumn (Figure 2).

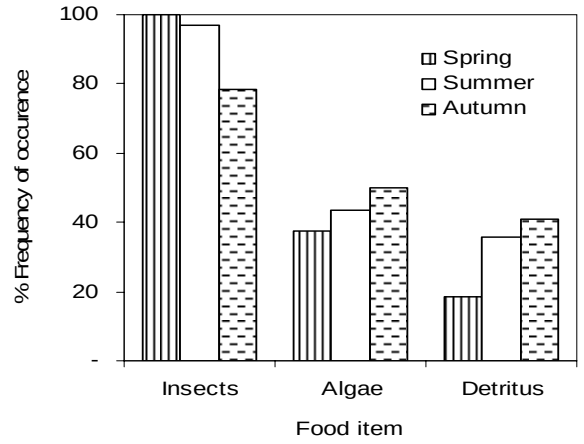


Figure 2. Seasonal changes in frequency of occurrence of various food items in the diet of *R. brunneus* 'orange' in Ado River.

There was a similar trend for food items' contribution to the diet: decreasing insect contribution (over 75% of the diet in spring, about 45% by autumn) but increasing algal and detrital contributions from spring to autumn (Algae: about 16% and 44% of diet in spring and autumn, respectively; Detritus: about 3% in spring and 10% in autumn) (Figure 3).

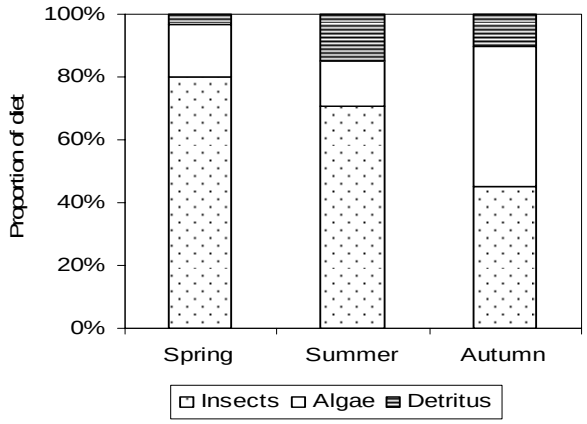


Figure 3. Seasonal changes in overall diet composition of *R. brunneus* 'orange' in the Ado River.

Diet composition significantly varied with season (NP MANOVA, Bray-Curtis F = 8.598, p<0.005). Insects made up about 79.26% ± 9.49 of individual fish diets in spring and this changed slightly to 76.25% ± 5.35 by summer (Mann-Whitney U test, Ub = 1498, P>0.05). From summer to autumn, however, this contribution decreased significantly to 53.82% ± 8.20 of diet (Mann-Whitney U test, Ub = 1918, P<0.01). Insect contribution to diet in spring was also significantly higher than that for autumn (Mann-Whitney U test, Ub = 557.0, P<0.01).

Algal proportion of individual diets remained stable between spring (11.13% ± 7.40) and summer (9.59% ± 3.23) (Mann-Whitney U test, Ub = 1578, P > 0.05) but significantly rose almost three fold from summer to autumn (28.04 ± 9.63) (Mann-Whitney U test, Ub = 2145, P < 0.05). Although mean algal proportion of individual diets was lower in spring than in autumn this difference was insignificant (Mann-Whitney U test, Ub = 668.5, P > 0.05).

Changes in detrital matter in the diet were not significant between spring (6.12 ± 6.29) and summer (13.93 ± 4.88)(Mann-Whitney U test, Ub =1322, P > 0.05), between summer and autumn (15.37 ± 7.11) Mann-Whitney U test, Ub = 2569, P > 0.05 as well as between spring and autumn (Mann-Whitney U test, Ub = 652.0, P > 0.05) (Figure 4).

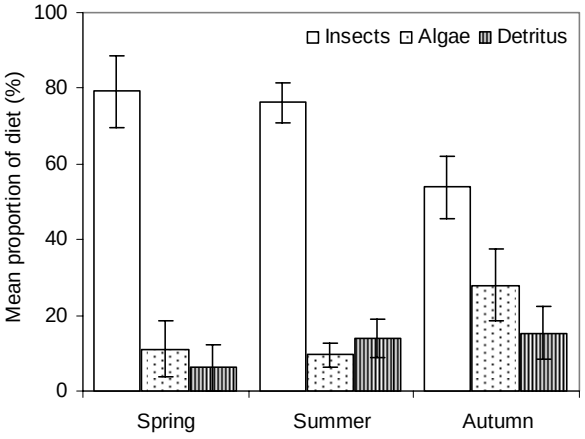
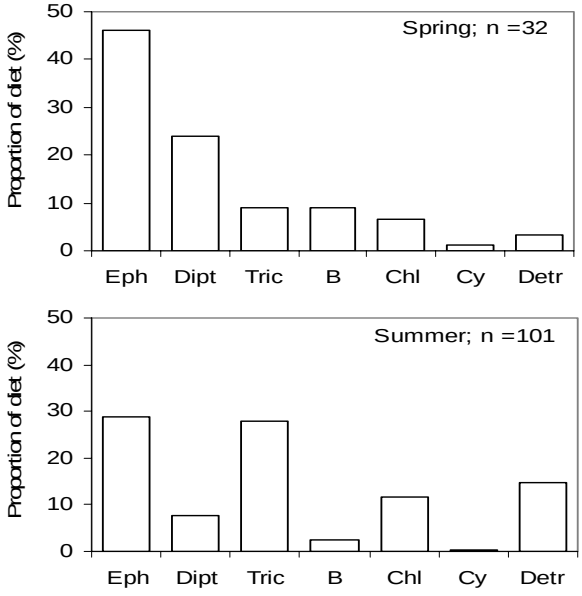


Figure 4. Changes in mean contribution to the diet of the main food items in *Rhinogobius brunneus* 'orange' in Ado River (Bars indicate 95% CL).

The relative importance of the major food items varied from season to season. Food item relative dominance was uncorrelated between spring and summer (Spearman's rank correlation coefficient r_s = 0.57, n = 7, p>0.05), between summer and autumn (r_s = 0.43, n = 7, p>0.05) and between spring and autumn diets (r_s = 0.00, n = 7, p>0.05). While the most

important food items in spring were Ephemeroptera and Diptera (together about 70% of the diet), Ephemeroptera, Trichoptera and detritus formed the bulk of the diet (72%) in summer. In autumn, a combination of Ephemeroptera, Chlorophyta, Cyanophyta and Trichoptera dominated the diet in that order (about 68% of diet). The importance of Ephemeroptera in the diet steadily declined from spring (46% of diet) through summer (29%) to autumn (21%)(Figure 5). The same pattern was true for Diptera (24% of diet in spring, 8% in summer and 7% in autumn). Trichoptera contribution to the diet trebled from spring (9% of diet) to summer (28%), but dropped again (11%) by autumn. Chlorophyta algae progressively increased in diet from spring (7%) through summer (12%) to autumn (21%). Cyanophyta algae made up just about or less than 1% of diet in spring and summer but by autumn it contributed 15% of the diet. Detrital matter was also less important in spring (3% of diet) than it was in summer (15%) and autumn (10%). Diet composition became increasingly diverse from 1.61 in spring to 1.77 in summer to 2.15 by autumn. The evenness of the abundance of food categories in the stomach as estimated by Pielou's index rose from 0.67 in spring and summer to 0.87 by autumn. For instance, only two food items dominated the diet with a combined 70% contribution by weight in spring (Ephemeroptera and Diptera), but a similar proportion of diet (68%) in autumn consisted of four almost evenly represented food items (Ephemeroptera, Chlorophyta, Cyanophyta, and Trichoptera)(Figure 5).



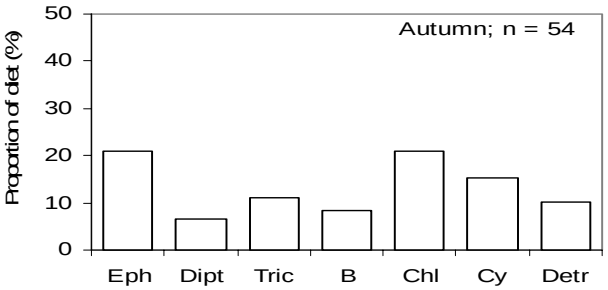


Figure 5. Seasonal changes in the diversity of diet of *Rhinogobius brunneus* 'orange' in Ado River (Eph: Ephemeroptera, Dipt.: Diptera, Tric.: Trichoptera, B.: Bacillariophyta, Chl.: Chlorophyta, Cy.: Cyanophta, Detr.: Detritus).

b. ***Changes in feeding intensity and condition factor***

The proportion of empty stomachs more than trebled from 6% in spring (n = 34) and 5% in summer (n = 107) to about 28% by the autumn season (n = 75). Stomach fullness of adult *R. brunneus* (orange) changed from 27.27 ± 4.88 (n = 32) in spring to 24.85 ± 3.03 (n = 101) by summer but this decrease was insignificant (Mann-Whitney U test, Ub=1405, p>0.05). Between summer and autumn, however, stomach fullness dropped significantly to 13.06 ± 2.59 (Mann-Whitney U test, Ub=1396, p<0.05)(Figure 4). Stomach fullness was also higher in spring than in autumn (Mann-Whitney U test Ub=340.5, p<0.001) (Figure 6).

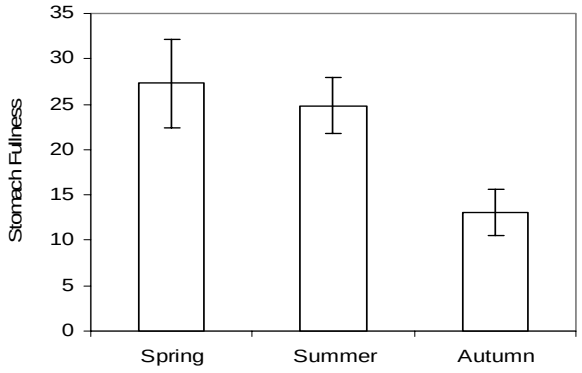


Figure 6.
Seasonal changes in the stomach fullness of *Rhinogobius brunneus* 'orange' in Ado River (Bars indicate 95% CL).

Condition factor was higher in spring (2.58 ± 0.13) than in summer (2.26 ± 0.05)(Mann-Whitney U test, Ub = 0.00012, p<0.001), in summer than in autumn (1.74 ± 0.04)(Mann-Whitney U test, Ub = 0.0001, p<0.001) and was also higher in spring than in autumn (Mann-Whitney U test, Ub = 665.0, p<0.001)(Figure 7).

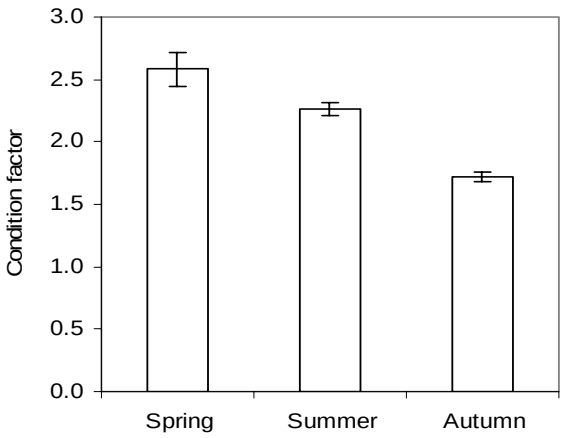


Figure 7. Seasonal changes in condition factors of *Rhinogobius brunneus* 'orange' in Ado River (Bars indicate 95% CL).

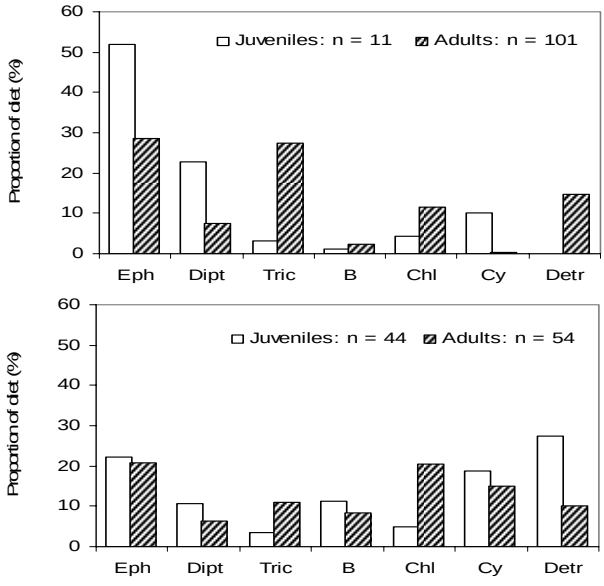


Figure 8. Ontogenetic diet differences in *Rhinogobius brunneus* 'orange' in Ado River in summer (above) and autumn (below) (Eph: Ephemeroptera, Dipt.: Diptera, Tric.: Trichoptera, B.: Bacillariophyta, Chl.: Chlorophyta, Cy.: Cyanophta, Detr.: Detritus)

3. Size-related variations in the diet

a. Diet composition

Diet composition significantly varied with fish size both in summer (Juvenile vs adult, NP MANOVA, Bray-Curtis F = 9.372, $p<0.01$) and in autumn (Bray-Curtis F = 6.621, $p< 0.01$). Adult fish diet was also more diverse than that of juveniles both in summer (Index of diversity: 1.77 vs 1.55) and in autumn (Index of diversity: 2.15 vs 1.80)

While insects made up over 84% of the juvenile diet (by weight) in summer, they contributed only up to about 70% to the adult diet. No juvenile fish in summer, but about 36% of adult fish stomachs had detritus (about 15%). In autumn adults ate more algae (44% of diet) than juveniles (35% of diet) and less detritus (10% of diet) than juveniles (27% of diet).

The relative dominance of individual food items in the adult diet was not correlated with their comparative importance in juveniles both in summer (Spearman's rank correlation coefficient, $r_s = 0.11$, n = 7, $p>0.05$) and in autumn ($r_s = 0.11$, n = 7, $p>0.05$). The three most important food items (in order of decreasing importance) for juveniles in summer were Ephemeroptera, Diptera and Cyanophyta while Ephemeroptera, Trichoptera and Detritus featured most principally in adult stomachs. In autumn the three most prominent food items in the juvenile diet were Detritus, Ephemeroptera and Cyanophyta while adults ate Ephemeroptera, Chlorophyta and Cyanophyta in that order (Figure 8).

b. Feeding intensity

In summer, all juvenile fish had food in their stomachs (n = 11) while 5% of adult stomachs were empty (n = 107). About 8% of juvenile fish were empty in autumn (n = 48) but more than three times that proportion of adult fish stomachs (28%) had no food (n = 75). Juvenile stomachs were fuller (58.9 ± 16.89) than adult fish stomachs (24.8 ± 3.03) (Mann-Whitney U test Ub = 143.5, $p<0.001$) in summer. In autumn juvenile fish also had fuller stomachs (17.3 ± 3.37) than adult fish (13.0 ± 2.59) (Mann-Whitney U test, Ub = 928.5, $p<0.05$)(Figure 9)

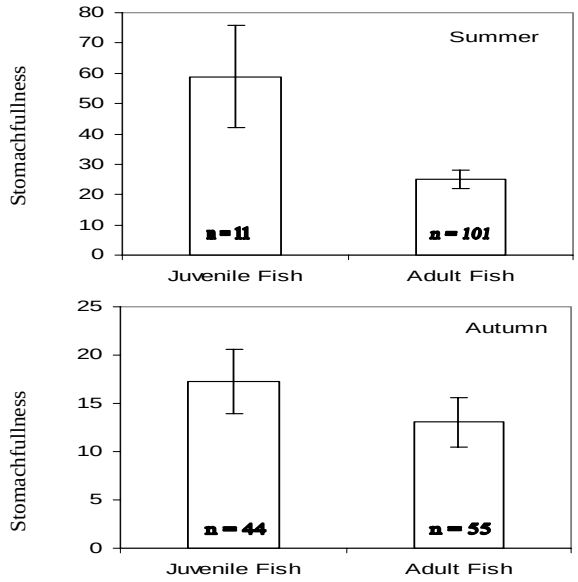


Figure 9. Ontogenetic differences in the stomach fullness of *Rhinogobius brunneus* '(orange') in Ado River. (Bars indicate 95% CL).

Discussion

Feeding behaviour of *Rhinogobius brunneus* 'orange' was observed using aquascopes placed on the water surface and steadied by hand. When cropping algae the fish fed mostly on slanting surfaces of stones, briefly detaching from the substrate and assuming a standing-on-head posture, making several snap-and-tear attempts at the algal growth across the span of the angled stone surface. Insects were collected from stone surfaces and the flowing water column. Sometimes fish made abrupt dashes into stone crevices to pick up insects.

Rhinogobius brunneus 'orange' in the Ado River exploits a wide spectrum of food items, in changing proportions at different times of the year, with insects being dominant in the diet. Kawanabe (1959) found that whereas these gobiid fishes mainly feed on aquatic insects in Ukawa River, algae dominate their diet in Katuragawa River in Kyoto prefecture. The diet pattern of gobies in this study resembles that of gobies in the Ukawa River. Tendencies for euryphagy and diet plasticity are common in ubiquitous riverine fishes (Lowe-McConnell, 1987) and most river-dwelling fishes in Japan are no exception (Kawanabe 1959, Osugi *et al.,* 1998). Unlike continental rivers, those in Japan are generally short and in steep gradients (Osugi *et al.,* 1998) and the fluvial environment is unstable (Kawanabe 1959). Diet generalisticity could serve as a suitable living strategy as opposed to strict trophic specialisation in such erratic temperate rivers, where food conditions may be suddenly altered by the fluctuating habitat conditions (Kawanabe 1959, Lowe-McConnell 1987, Osugi *et al.,* 1998).

Diet composition showed increasing diversity from spring to autumn; few food items dominated the diets of spring and summer seasons while a wider scope of evenly represented food items typified the autumn diet. Diets may expand and contract according to the quality and availability of alternative foods (Hughes 1993). Scarcity of food resources leads to the diversification of diet composition (Carrason and Matallanas, 2001). Less profitable and hitherto unconsumed prey become more utilised as the density of more profitable prey declines (Wootton 1990). Diets therefore tend to be broader during lean seasons than during rich periods (Perry and Pianka 1997). The spring and summer seasons, with higher stomach fullness and less proportion of empty stomachs (than the autumn season) may correspond to rich periods in this study. Although euryphagic in general, *R. brunneus* 'orange' therefore seems to have the capacity to opportunistically confine or expand its trophic repertoire. Such a capability to widen or restrict diets has been demonstrated in a related Japanese river-dwelling *Rhinogobius* species in line with ecological release (Osugi *et al.,* 1998).

Insect consumption was higher in spring and in summer than in autumn whereas algal consumption showed an opposite trend. This is probably due to changing availability and/or suitability of these food items. Food resources vary throughout the annual cycle (MacNally, 1995) and most fish species in turn modify their food habits in correspondence with this qualitative and quantitative seasonal change of food organisms (Sunaga 1964). Aquatic insect juveniles that have a larval or nymphal stage in their life cycle leave the water upon becoming adults (Merritt and Cummins 1978, Wootton 1990, Watanabe *et al.,*1999). In parts of Japan this aquatic insect emergence takes place from late June to late August (Watanabe *et al.,* 1999). The riverine aquatic insect biomass therefore dwindles towards autumn (Yamamoto *et. al* 1988). Such a pattern in the aquatic insect life cycle could decrease insect prey availability and deprive the insectivorous *R. brunneus* 'orange' of a valuable food resource and in turn affect its overall food habits. Optimal diet theory predicts a forager to always consume the most profitable food type and resort to successively less profitable types only when encounter rates with higher-ranking types fall below critical levels (Hughes, 1993). In this way predators can take advantage of booming abundance of a rewarding food resource (Magalhaes 1992, Dawe *et al.,* 1998). In another Japanese stream, as aquatic insect biomass decreases from spring to autumn so does the stomach fullness of insect-preying fish, *Cottus hangiongensis* and *C. nozawae* (Yamamoto *et. al* 1988). *Rhinogobius brunneus* 'orange' may have fed on more aquatic insects in spring and summer than in the autumn (when instead more algae and detritus were consumed) to maximise use of a plentiful protein-rich food resource.

Changes in condition factor may allude to shifts in feeding intensity, with high condition factors indicating an abundance of food supporting both somatic and gonadal growth (Wootton 1990, Moyle *et. al.* 1996). Feeding intensity of *R. brunneus* 'orange' progressively decreased from spring through summer to autumn as revealed by increased number of empty stomachs and decreased stomach fullness and condition factor. Seasonal water temperature changes in temperate areas affect activity patterns of biota in the aquatic ecosystem (Worobec 1984, Watanabe *et al.,* 1999). The emergence of the mayfly, *Ephoron shigae*, in Japanese rivers is closely related to temperature changes (Watanabe *et al.,* 1999). Feeding in natural fish populations may correspondingly decrease or totally cease with lowering temperatures especially

in autumn and winter and only resume with higher spring temperatures (Worobec 1984). There is a possibility of the trophic ecology of *R. brunneus* 'orange' in this temperate river being affected by such temperature changes, although lack of samples from all months makes elucidation of this assumption difficult. Gonadal somatic index values are also unavailable in this study. However, the reproductive season of *R. brunneus* 'orange' extends from March to August when gonadal activity is generally high, with a peak in April (Katoh 1996). Both feeding intensity and reproductive activities may thus be driving changes in condition factor from spring across summer to autumn.

Juvenile lake-river migration of *R. brunneus* 'orange' occurs mostly in summer (Yuma *et al.,* 2000), a period of high quality food (insects) and high feeding activity in this fish (this study). As the juvenile stage is a vulnerable one in a fish's life cycle (Wootton 1990) this timing of reproduction and migration will enable the young to grow fast into size classes less vulnerable to predation (Wootton 1990) and is thus advantageous.

This study revealed a size-related difference in the diet of *R. brunneus* 'orange'. The relative importance of specific food items in juveniles was not correlated with their importance in adults. Moreover, smaller fish had fuller stomachs and a less diverse diet than adults. Ontogenetic changes in diet are common in many types of animals (McCormick 1998, Miranda and Gu 1998). The spectrum of prey sizes ingested by fish widens with an increase in the size of the predator (Kislalioglu *et al.,* 1976, cited in Wootton 1990). Apart from size-related morphological and maturational changes (e.g. increase in mouth size, improvements in locomotory and sensory abilities) ontogenetic differences in foraging microhabitat use may also explain this difference (Wootton 1990, Magalhaes 1992, Moyle *et al.,* 1996). For instance, juveniles ate almost twice as much Ephemeroptera (over half of the diet) as adults in summer. Some Ephemeroptera species particularly associate with river margins (Ormerod 1988). Younger (and usually upstream-migrating) *R. brunneus* 'orange' gobies mostly inhabit river margins at sites shallower than 20cm and do not occur in the deeper middle of the stream flow where current velocity is higher (Yuma *et al.,* 2000).

In conclusion *R. brunneus* 'orange' is a euryphagic fish that seasonally and ontogenetically confines or expands its trophic repertoire. It feeds more in spring and summer than in autumn and matches its reproductive season with times of plenty and high feeding intensity. *R. brunneus* 'orange' thus demonstrates a considerable level of adaptation to living in an unstable temperate riverine environment.

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