Journal of Fisheries International 2 (1): 91-98, 2007

The Effect of Two Frozen Natural Foods on the Routine Metabolism and Ammonia Excretion of the Pacific Seahorse Hippocampus Ingens

[1]María Idalia Sandoval-Muy and [2]Benjamín Barón Sevilla
[1]Centro de Estudios Superiores del Estado de Sonora, Periférico Sur Carretera a Huatabampo,
Navojoa, Sonora México A.P. 455
[1]Departamento de Acuicultura, Centro de Investigación Científica y Educación Superior de Ensenada,
Km 107 Carretera Tijuana-Ensenada, Ensenada, Baja California, México

Abstract: Effects of diet on metabolism of cultured aquatic organisms are reflected both in oxygen use and nitrogen excretion. The objective of this work was to study the effects of two frozen natural foods on the oxygen consumption and the ammonia excretion of the Pacific seahorse, Hippocampus ingens, which is distributed throughout the tropical American Pacific Ocean. Routine metabolism and ammonia production were measured in individuals with an average weight of 34.9±1.64 g, at 22°C and 35 ‰ salinity. Seahorses were fed either of two frozen foods, 1) Mysis relicta and 2) Artemia franciscana. Routine metabolic rates of fasted animals previously fed the two diets were 0.037±0.016 and 0.036±0.012 mg O_2 h^{-1} g wet wt and ammonia excretion of 0.0031±0.0023 mg NH_3-N h^{-1} g wet wt and 0.0021±0.0006 mg NH_3-N h^{-1} g wet wt, respectively. Seahorses processing a recent meal of M. relicta had a significant increase in oxygen consumption rate and ammonia excretion (0.058±0.021 mg O_2 h^{-1} g wet wt and 0.0076±0.003 mg NH_3-N h^{-1} g wet wt ., respectively). This effect was somewhat less evident for seahorses fed A. franciscana (0.050±0.019 mg O_2 h^{-1} g wet wt and 0.0025±0.001mg NH_3-N h^{-1} g wet wt). We recommend a mixture of these diets for maintenance of Pacific seahorses in captivity.

Keywords: Seahorse, hippocampus ingens, routine metabolism, oxygen consumption, ammonia excretion

INTRODUCTION

The Syngnathidae family comprises a group of bony fishes known as pipefishes, sea-dragons, pipehorses and seahorses with a very peculiar appearance. The seahorses have an upright posture, a head bend at angle, a tubed snout with a small mouth at the tip and strong prehensile tail to grab onto things (Kuiter, 2000). All seahorses have been grouped into the genus Hippocampus with 33 known species distributed in tropical and temperate regions of the world, between 50°N and 50°S (Lourie *et al*., 2004).

Studies related to the biology the seahorses have been partially motivated by the high value of this organism in the Asian market where they are commonly used as ingredients in the natural medicine and by the high demand of this as ornamental fishes for the aquarium trade. At least 32 countries around the world are involved in the seahorses trade. The annual demand of the Asian countries, without considering Japan, Korea, Malaysia and Singapore, is around 45 tons of dry fish. The extraction of seahorses from their natural environment could have serious effects on their natural populations (Vincent, 1996). Unfortunately, information related to the biology and ecology of this group of fishes is still scarce and fragmentary; and is concentrated mainly on the species of commercial importance due to their trade as ornamental species and those used in Chinese traditional medicine.

Seahorses are difficult to maintain in captivity. Recently, it has been possible to cultivate *H. abdominalis* at commercial scale (Forteath, 1996, 1997, 2000). Although the reproductive strategies of the Syngnathids have been studied extensively (Vincent *et al*., 1992), the scarcity of information about nutrition, reproduction and biology on most species, including the Pacific seahorse, curbs the development of an adecuate culture protocol. In particular, the nutritional requirements and the appropriate food types have been identified as the main obstacles for the successful culture at a commercial scale (Binsheng, 1992), Some advances have been achieved using live food (Giwojna, 1996), but quality and availability are unpredictable. Woods and Valentino (2003), recommend the use of frozen mysids as a better alternative for culture and maintenance of adult seahorses, since it are an important component in the seahorse natural diet and have been found to increase survival under captivity. Brine shrimp, is not recommended as the only food source for the adults seahorses (Woods, 2002).

Corresponding Author: María Idalia Sandoval-Muy C.I.C.E.S.E., Department of Aquaculture, P.O. Box 434844,
San Diego, CA 92143-4844, USA

The metabolic rates of many temperate and tropical fish species of economic importance have been studied to know about the influence of different environmental parameters, such as temperature, salinity and food in metabolic rate (Brett, 1972; Thutrson and Gehrke, 1993; schurmann and steffensen, 1997; Lyytikainen and jobling, 1998; Clarke, 1999; Claireaux and Lagardere, 1999; Chipps *et al.*, 2000; Mallekh and Lagardere, 2002; Kajemura *et al.*, 2004). Interest I n research and culture of the seahorses has increased, studies about the effect of the diet on their metabolism are scarce (Masonjones, 2001). Recently, the potential for the cultivation of Hippocampus ingens has been identified in Mexico, because at the large size reached by the adults of this species (up to 300 mm) (Vincent, 1996; Project Seahorse, 1999; Lourie *et al.*, 2004) and the good trade value currently obtained in the Chinese traditional medicine (Lourie *et al.*, 2004).

From a theoretical point of view, it is relatively easy to understand how an organism uses the energy obtained from ingested food, at the experimental level is difficult (Johling, 1985). There is no consensus on the methodologies and definitions related to the different metabolism terms (Jobling, 1994; Lucas, 1996), in this study we used the concept of routine metabolism defined by Beamish and Mookherjii (1964) and Calow (1985), as oxygen consumed by an organism in a state of digestive rest and its activity limited to spontaneous movements (Beamish and Moookherjii, 1964). This may account up to 50% of the active metabolism (Job, 1955).

The objective of this research is to study the effect of two natural frozen foods over routine metabolism and ammonia excretion of the Pacific seahorse Hippocampus ingens.

MATERIALS AND METHODS

Adult seahorses were collected in the Bay of Mazatlán, Sinaloa, México (23°15'N and 106°25'W) using scuba diving. Seahorses were transported to the Laboratory at the Aquacutlure Department, CICESE in polyethylene bags with seawater at 24 C satured with pure oxygen. A total of 24 seahorses (9 females and 15 males), with an average length of 195.7±3.15 mm and an average weigth of 34.9±1.64 g, were divided into six groups.

Each group of four seawhorses was placed in a 500 L circular flat bottom tank and each seahorse was confined within the tank in a 20 L white bucket that have two 15×20 cm windows covered with a 2 mm mesh to allow water circulation and homogenous water quality. Temperature and salinity were kept to 22±1C and 35±1‰, respectively.

Seahorses from three tanks were fed with frozen Artemia franciscana (Salt Lake City) and the three remaining with Mysis relicta (Piscine Energetics, Canada). In both study, the ration was equivalent to 4% of the wet biomass per day and provided in two equal parts, one at 0900 and the other at 1700. The feeding trial with both experimental diets last 20 days. Each morning before feeding and 2 h after feeding uneaten feed was siphoned out of the tank. Artemia and the mysid rations were thawed before feeding at room temperature.

Proximate analysis of the food supplied, was determined using routine laboratory techniques. Crude protein was determined using the Kjeldahl method (Official Mexican Norm NOM-129-SSA1-1995) and total lipids with the Soxlet method (Official Mexican Norm NOM-086-SSA1-1994). Moisture content was determined after incubating the samples for 24 h at 60°C and ash content was measured after incinerating the sample at 460°C for 8 h. The Nitrogen Free Extract (NFE) was estimated as the difference between sample dry weight and the sum of proteins, lipids and ashes.

A semiclosed respirometer that consisted on a rectangular acrylic tank of 220 L with 14 respirometric chambers (14 cm diameter and 23 cm height) was used to measure the oxygen consumption and the ammonia production (Fig. 1) (Buckle *et al.*, 2003). The seahorses were transferred individually to each chamber of 3.55 L. Temperature and salinity inside the chambers were maintained at the same values as in the acclimation tanks (22°C and 35‰).

Fourteen chambers were placed in the respirometer, two of them were used as control to measure oxygen consumption of microorganisms in the seawater. To eliminate the stress effect caused by manipulation and the specific dynamic action of the food, the seahorses remained in the chambers for 24 h in recirculating water, before performing any measurements. Afterwards the initial concentration of oxygen was measured with YSI oxygen meter (Model 57), provided with a polarographic sensor. The chambers were closed for 120 min, disolved oxygen saturation were mainted to 84±6.9% in both experiments. A water sample from each respirometric chamber was taken after 120 min to measure the final oxygen concentration. Seahorse oxygen consumption was calculated as the difference between the initial and the final oxygen values and was expressed in mg O_2/seahorse/hour. The average oxygen consumption of both controls was used to correct the oxygen consumption of every organism.

Nitrogen excretion and oxygen consumption measurements were carried out simultaneously. To measure the Total Ammonia Concentration (TAN), the indophenol-blue method was used (Rodier, 1981). The concentration of TAN was measured with a spectrophotometer (Shimadzu, UV-1201) at a wavelength of 640 nm. The nitrogenous excretion value was calculated as the difference between final and initial TAN concentrations.

Fig. 1: Diagram of the flow-through respirometer used to measure the oxygen consumption and the ammonia excretion of Hippocampus ingens. 1, Respirometer; 2, Pump; 3, Heater; 4, Electric control; 5, Water distributor; 6, Respirometric chamber; 7, Air

Five measurements were performed during 18 h, the first three under digestive rest conditions. The other two measures were made 1 and 2 h after feeding to estimate the increment in the metabolic rate due to food consumption (specific dynamic action).

Fish were weighed and measured individually at the beginning of the experiment. To estimate routine metabolism, wet weights of the organisms were used, because they could not be sacrificed to measure the dried weight. All data were analyzed using one way Analysis of Variance (ANOVA) (Statistica, Statsoft Inc.) at a level of significance of $p \leq 0.05$).

RESULTS

The proximal composition of the two frozen foods used in this study revealed that the content of proteins and lipids of the Mysis relicta is 1.5 and 3.4 times higher than Artemia franciscana, respectively. Lipid:protein ratio is 0.78 and 0.34 for M. relicta and A. franciscana respectively. Ash content of A. franciscana is higher than that content of M. relicta (Table 1).

In their natural environment, seahorses remain a long time attached by their tails to macroalgae or corals. The same behaviour was observed in respirometric chambers since seahorses were fastened by their tails to the glass tube that served as water entrance into the respirometric chamber. During routine metabolism measurements, spontaneous swimming movements and slight movements of the dorsal fin were observed. However, the time that seahorses spent in these activities were not quantified.

Table 1: Proximate composition of frozen food used in the experiments

	Mysis relicta (%)	Artemia franciscana (%)
Proteins	57.23	39.25
Lipids	44.82	13.37
Carbohydrates	0	26.74
Moisture	75.59	87.21
Ash	6.76	20.64

Table 2: Rates of oxygen consumption and ammonia excretion in Hippocampus ingens in digestive rest condition and fed two frozen diets. N = sample size

Food	Oxygen consumption (mg O_2 h^{-1} g wet wt)	Nitrogen excretion (mg NH_3-N h^{-1} g wet wt)
Mysis relicta	0.037±0.016 n = 36	0.0031±0.0023 n = 20
Artemia franciscana	0.036±0.012 n = 23	0.0021±0.0006 n = 12

No significant differences between the rates of oxygen consumption (p = 0.8) and ammonia excretion in routine conditions (p = 0.165) were founded when the seahorses were fed with the two diets (Table 2).

Oxygen consumption rates of seahorses fed with Mysis relicta was 0.037±0.016 mg O_2 h^{-1} g wet wt (Fig. 2). After the food ration was provided, all seahorses began to eat and a 38% increment in the oxygen consumption rate was observed (0.058±0.021 mg O_2 h^{-1} g wet wt) and it was significantly different (p = 0.0008) from the digestive rest period. This effect is less evident with organisms fed with A. franciscana, (Fig. 3). Although significant differences were observed (p = 0.0009), only a 28% increment oxygen consumption, which might indicates that some organisms did not eat. When the ration of Artemia was given, only 3 of the 12 organisms displayed head movements. Head movements are characteristic of feeding activity. Feces excretion was observed after 9 h.

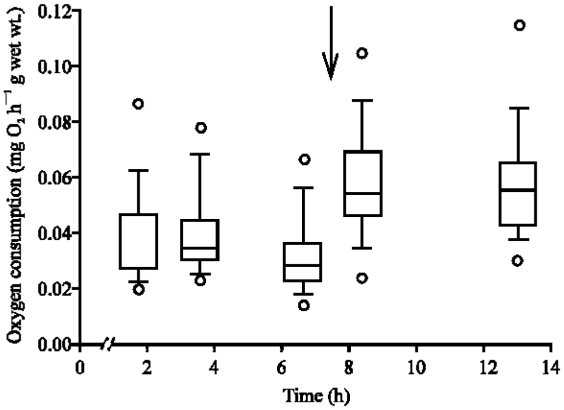

Fig. 2: Oxygen consumption of the seahorse Hippocampus ingens, fed on Mysis relicta. The first three boxes represent the oxygen consumption in fast condition and the last two boxes in post-feeding condition. The horizontal line within the box represents the median, the horizontal lines at the end of the vertical lines represent 95% of the data and the circles represent extreme values. The arrow represents the time when the seahorses were fed

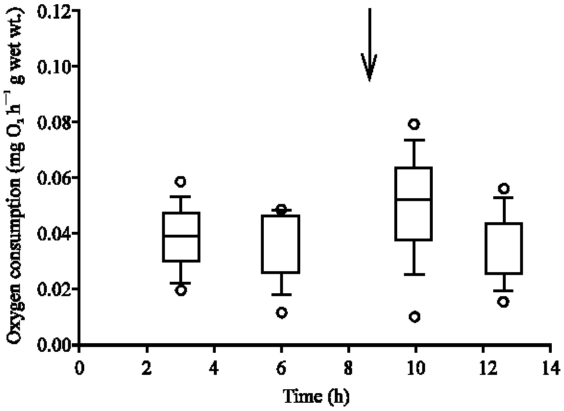

Fig. 3: Oxygen consumption of the seahorse Hippocampus ingens, fed on Artemia franciscana. The first two boxes represent the oxygen consumption in fast condition and the last two boxes in post-feeding condition. The horizontal line within the box represents the median, the horizontal lines at the end of the vertical lines represent 95% of the data and the circles represent extreme values. The arrow represents the time when the seahorses were fed.

After food ingestion, the rate of ammonia excretion of seahorses fed with Mysis relicta was 3 times higher (0.0076±0.003 mg NH3-N/h g wet wt) than the rates mesured in seahorses fed with Artemia franciscana (p = 0.0001) (Table 3).

Table 3: Rates of oxygen consumption and ammonia excretion in Hippocampus ingens in post-prandial conditions and fed two frozen diets N = sample size

Food	Oxygen consumption (mg O_2 h^{-1} g wet wt)	Nitrogen excretion (mg NH_3-N h^{-1} g wet wt)
Mysis relicta	0.058±0.021 n = 12	0.0076±0.003 n = 9
Artemia franciscana	0.050±0.019 n = 12	0.0025±0.001 n = 12

DISCUSSION

In the natural environment seahorses feed on crustaceans, in particular mysids are important components of their diet (Woods, 2002). This has important implications for the culture of this organism in captivity, since reports indicate low success in the culture of seahorses using formulated diets, due to their preference for live food and to the deficient quality of the frozen or formulated diets used so far (Woods, 2003). They are several succesfull laboratory studies with early juveniles seahorses fed with mysids and frozen copepods (Payne and Rippingale, 2000; Woods, 200, 2003). In this study, adult seahorses, Hippocampus ingens, collected from the wild and acclimated to laboratory conditions were trained to feed on frozen mysids and Artemia successfully.

Studies on the metabolic rates of members of the Syngnathidae family are very scarce (Masonijones, 2001; Leiner, 1937) and in particular *H. ingens* have not been studied to date. In addition no information on the interval of the thermal tolerance or on the preferred temperature of this species has been published. Therefore a temperature of 22°C was choosen for this study, considering the temperature were seahorses are naturally distributed (i.e., 5 and 40°C). *H. ingens* it is distributed from Northern Peru to San Diego (Miller and Lea, 1972) where the temperature beneath the sea can vary from 18 to 28°C (Tchernia, 1980) and in laboratory conditions, the maintenance condition for *H. ingens* was from 15 to 25°C and the optimun is of 23°C (Gomezjurado and Bull, 2002).

In this study, oxygen consumption rates and nitrogen excretion, measured in seahorses fed with mysids or Artemia, were not significantly different. However, according to Jobling (1994) these rates in the fish and other animals are influenced by diet quality and several other factors that conform the ecological ambient of the species. The results of the studies performed with starved domestic animals suggestes that the metabolic rate can be affected by the previous nutritional regime of the organism. In our experimental results do not show a significant difference in the routine metabolism of the seahorses fed with mysids or Artemia as could be expected considering that the biochemical composition of the dry items are different. The ratio lipids: Proteins was 0.78 and 0.34, respectively. It is probable that the cost of the basal and routine metabolism are not substantially different between the seahorses fed with two diets and in the short term (20 days) the food contribute the necessary energy for his metabolism.

In fish, metabolic rates are variable, Thurston and Gehrke (1993) classified the metabolic routine rates of 237 species of fish, from a database of 3573 at a standard temperature of 20°C. A significant relation was found between metabolic consumption and mass ($r^2 = 0.838$; ln to = -0.754: b = 0.646). An hipotetic average fish of 35 g has an oxygen consumption, of 4.66 mg O_2 h^{-1} that is equivalent to Specific Routine Metabolic Rate (SRMR) of 131.3 mg O_2 kg^{-1} h., because metabolic rate of fish depends of different factors like temperature, salinity, corporal mass and activity level, thus, metabolic routine rates measured in different species of tropical fish, vary considerably. Mugil cephalus with a mass of 33.5 g has a metabolic rate of 2.04 mg O_2 h^{-1} to 13°C (Marain, 1978) and in according with Thurston and Gehrke (1993) the standardized rate to 20°C corresponds to 3.9 mg O_2 h^{-1} (SRMR = 116.4 mg O_2 kg^{-1} h), in contrast, Anabas testudineus with 30.5 g of body mass and water temperature of 28°C, have a metabolic rate of 0.24 mg O_2 h^{-1} (Panigrahi *et al.*, 1984) and in agreement with Thurston and Gehrke (1993) at 20°C, the metabolic rate is 0.12 mg O_2 h^{-1} (SRMR = 393.4 mg O_2 kg^{-1} h). In Esox lucius with of 35.5 g of body mass, the routine rate at 21.3 C is 0.75 mg O_2 h^{-1} (Petit, 1973), which is equivalent to a SRMR of 21.1 mg O_2 kg^{-1} h (Thurston and Gehrke, 1993) and is lower than *H. ingens*, with an average body mass of 34.9±1.64 g and a routine metabolic rate of 1.27 mg O_2 h^{-1} (average SRMR = 36.5 mg O_2 kg^{-1} h).

The differences in metabolic rate of H. inges probably reflects the fact that this species is an ambush predator that use their vision to search for prey with a relatively low level of spontaneous activity (Garrick Maidment, 1997; Lourie *et al.*, 1999). In contrast, marine herbivore and omnivore organisms show a more active feeding activity. Seahorses have a high mimicry capacity that can make them difficult to find since they are attached to a substrate similar to the color and appearance of their skin (Vincent, 1996; Project Seahorse, 1999; Lourie *et al.*, 2004).

In routine conditions, which involve a condition (state) of digestive rest and limited spontaneous activity, oxygen consumption and ammonia excretion depend mainly on temperature and time that the organism remains without feeding, in such a way that it will use his own reserves as metabolic substratum (i.e., glycogen in the liver, adipose tissue, free amino acids or muscular proteins) (Wood, 1993; Jobling, 1994, 1996). The quality of the diets used in this study were different in proximal composition (Mysis relicta content of proteins was 1.5 higher than Artemia), but not difference was observed in rates of ammonia excretion of *H. ingens* under routine conditions, this result can reflect the fact that both diets provide the necessary nutrients and energy.

In routine conditions, marine fish of different taxonomic groups ranging from 4 to 1,000 g of body mass, the smaller organisms (between 4 and 20 g of weight) have the largest rates of ammonia excretion (Walsh *et al.*, 2001). This values range from 0.0083 to 0.0095 mg NH_3-N h^{-1} g for Bathyagonus nigripinnus and Cymatogaster aggregatus, respectively. In organisms with larger weights like Ophiodon elongatus (807-950 g of body mass), the ammonia rate excretion were smaller (0.0012 mg NH_3-N h^{-1} g). Ammonia excretion rates for the other marine species like Hippoglossoides elassodon (147-242 g) has 0.0018 mg NH_3-N h^{-1} g and Icelinus tenuis (Walsh *et al.*, 2001) with a similar weight to seahorses used in this study (28-46 g), ammonia excretion rates is 47% higher than that of *H. ingens.* We would expect a lower excretion rate even though both fish are of the same size, because the temperature used to determine ammnonia excretion of *I. tenuis* was 9°C lower that used with *H. ingens* (22°C).

The SDA represents the energy expense associated with the ingestion, digestion and assimilation of food, as well as the synthesis and deposition of proteins (Jobling, 1983, 1985; Conceicao *et al.*, 1998). Fish, as well as other organisms, increase their oxygen consumption and nitrogenous excretion after feeding (Jobling, 1981; Yager and Summerfelt, 1994; Hunt and White, 2001) and the increment can be 1.5-2.5 times higher than the pre-feeding levels (Jobling, 1981). Under conditions used in this experiment it was not possible to evaluate the amount of food consumed. In our expermient, the SDA of seahorses fed with mysids (with a percentage of protein greater than artemia) was 1.6 times higher than their routine metabolism and the ammonia excretion was three times higher, In contrast, a smaller difference was determined for the organisms fed with *A. franciscana.* This difference can be related to the fact that in respirometric chambers most seahorses did not ingest this food and can be explained in part by the appearance and structure of the food in the water column. Drastic changes ocurr to artemias as a result of the freezing and thawing processes that end up breaking the prey into pieces that the seahorses might not like. It is probable that the mysids, for their size and presentation, (8 mm in length and stay whole after thawed) were more attractive to the seahorses than the artemias. Artemia are widely used to feed seahorses, because they are easily cultured and are commercially available, but are not a natural prey item for seahorses (Wong and Benzie, 2003) and have been reported as difficult food to assimilate for *H. subelongatus* (Payne and Rippingale, 2000).

Artemia are easily cultivated from cysts and can provide nauplii of all sizes and developmental stages and have been used as live food for different marine organisms, including the larvae of marine fish and shrimp (Shapawi and Purser, 2003) and for juveniles and adults seahorses (Giwojna, 1996). Our results suggested that Artemia can be an acceptable alternative to feed adult seahorses and they can be maintained for a couple of months (Sandoval-Muy, unpublished data). However, considering that marine fish in general have a high requirement for Highly Unsaturated Fatty Acids (HUFA) (Sargent *et al*., 1997) and that artemia have a low content of HUFA with respect to the requirement by marine fish, artemia can be regarded as an inadequate food (Navarro *et al*., 1991).

Mysids are an important component in the natural foods of *H. abdominalis* (Woods, 2002). Some authors are hesitant to use of Artemia as the only food for the adults seahorses (Giwojna, 1996; Woods and Valentino, 2003) and recommend the use of frozen mysids as a better alternative for culture and maintenance of adult seahorses, since they have been found to increase survival under captivity. Juveniles of *H. abdominalis* fed live enriched Artemia nauplii, frozen mysids or a combination of both did not produce significant differences in length, wet weight, or condition factor (Woods and Valentino, 2003). These authors suggest using a mixture of mysids and live HUFA enriched Artemia as a good alternative for the maintenance of these organisms.

CONCLUSION

Frozen diets are a viable alternative for maintenance and adaptation to captivity of this species. Although no significant differences were detected in the rutine metabolism, when seahorses were feed either mysids or artemia we do not recommend the use of one type of frozen diet alone, but rather a mixture of both for the adecuate maintenance of the seahorse *H. ingens*.

ACKNOWLEDGMENTS

This study was supported by the Mexican Federal Goverment, through regular funds of the Centro de Investigación Científica y de Educación Superior de Ensenada (C.I.C.E.S.E.). The authors acknowledge to the Programa de Mejoramiento del Profesorado de Educación Superior (Superior Education Professor Improvement Program), Centro de Estudios Superiores del Estado de Sonora by the academic grant and also thanks to Dr. L. Fernando Bückle-Ramírez, Dr. J. Pablo Lazo Corvera and Dr. Manuel Segovia Quintero for the comments and criticism and to Francisco Valenzuela-Buriel for the respirometer schematic drawin.

REFERENCES

Beamish, F.W.H. and P.S. Mookherjii, 1964. Respiration of fishes with special emphasis on estandar oxygen consumption. I. Influence of weight and temperature on respiration in goldfish, *Carassius auratus* L. Can. J. Zool., 42: 161-175.

Binsheng, L., 1992. Research into the culture of *Hippocampus*. J. the Ocean Univ. Quingdao, Quingdao Haiyang Daoxoe Xoebao, 22: 39-44.

Brett, J.R., 1972. The metabolic demand for oxygen in fish, particularly salmonids and a comparisons with other vertebrates. Respiration Physiol., 14: 151-170.

Bückle, R.L.F., S.B. Barón, R.M. Hernández, M.A. Ledo, A.R. Solís, E.B. Pérez and S.A. Hernández, 2003. Sistema de temperatura, oxígeno y salinidad para la experimentación en ecofisiología. Hidrobiología, 13: 277-287.

Calow, P., 1985. Adaptive Aspect of Energy Allocation. In: Fish energetics. New perspectives. Tytler, P. and P. Calow (Eds.), Croom Helm. London, pp: 13-31.

Chipps, S.R., D.F. Clapp and D.H. Wahl, 2000. Variation in routine metabolism of juvenile muskellunge: Evidence for seasonal metabolic compesation in fishes. J. Fish Biol., 56: 311-318.

Claireaux, G. and J.P. Lagardère, 1999. Influence of temperature, oxygen and salinity on the metabolism of the European sea bass. J. Sea Res., 42: 157-168.

Clarke, R.D., 1999. Diets and metabolic rates of four Caribbean tube blennies, genus Acanthemblemaria (Teleostei: Chaenopsidae). Bull. Marine Sci., 65: 185-199.

Conceicao, L.E.C., Y. Dersjant-Li and J.A.J. Verreth, 1998. Cost of growth in larval and juvenile catfish (*Clarius gariepinus*) in relation to growth rate, food intake and oxygen consumption. Aquaculture, 161: 95-106.

Forteath, N., 1996. Seahorses, Hippocampus abdominalis in culture. Aust. Aquacult., 9: 83-84.

Forteath, N., 1997. The large bellied seahorse, Hippocampus abdominalis: A candidate for aquaculture. Aust. Aquacult., 11: 52-54

Forteath, N., 2000. INFOFISH International. Juala Lumpur. INFOFISH Int., pp: 48-50.

Garrick-Maidment, N., 1997. Seahorses: Conservation and care. England, Kingdom Books, pp: 48.

Gemezjurado, J. and C. Bull, 2002. Hippocampus ingens. In: Bull, C. and J.G. Shedd Aquarium Partnership for Marine Conservation, (Eds.). Seahorse Husbandry in Public Aquaria 2002 Manual. Chapters Contributed by Members of Syngnathid Discussion Group. John G. Shedd Aquarium Chicago, IL, USA, pp: 40-42.

Giwojna, P., 1996. Seahorse nutrition. Part I. Live Foods for Adults. Freshwater and Marine Aquarium Magazine (FAMA). 19(10): www.oceanrider.com/articles.asp.

Hunt von Herbing, I. and L.White, 2001. The effects of body mass and feeding on metabolic rate in small juvenile Atlantic cod. J. Fish Biol., 61: 945-958.

Job, S.V., 1955. The oxygen consumption of Salvelinus fontinalis. Ontario Fisheries Res. Lab. Publ., 73: 1-39.

Jobling, M., 1981. The influences of feeding on the metabolic rate of fishes: A short Rev. J. Fish Biol., 18: 385-400.

Jobling, M., 1983. Towards an explanation of specific dynamic action (SDA). J. Fish Biol., 23: 549-555.

Jobling, M., 1985. Growth. In: Tytler, P. and P. Calow (Eds.). Fish Energetics: New Perspectives. London: Cromm Helm, pp: 213-230.

Jobling, M., 1994. Fish Bioenergetics: Chapman Hall. London, pp: 309.

Jobling, M., 1996. Temperature and Growth: Modulation of Growth Rate via Temperature. In Wood, C.M. and D.G. McDonald, (Eds.). Global Warming: Implication for Freshwater and Marine Fish. Society for Experimental Biology, Seminar Series, Cambridge Univ. Press, Cambridge, 61: 225-253.

Kajimura, M., S.J. Croke, C.N. Glover and C.M. Wood, 2004. Dogmas and controversies in the handling of nitrogenous wastes: The effect of feeding and fasting on the excretion of ammonia, urea and other nitrogenous waste products in rainbow trout. J. Exp. Biol., 207: 1993-2002.

Kuiter, R.H., 2000. Seahorses, Pipefishes and Their Relatives. A Comprehensive Guide to Syngnathiformes. TMC Publishing, Chorleywood, UK.

Leiner, M., 1937. Die Atmung des kurzschnäuzigen Seepferdchens (Hippocampus brevirostris Cuv.) und seiner Embryonen und Jungfische. Ztsch. vergl. Physiol., 24:143-165.

Lourie, S.A., S.J. Foster, E.W.T. Cooper and A.C.J. Vincent, 2004. A Guide to the Identification of Seahorses. Project Seahorse and TRAFFIC North America. Washington D.C.: Univ. Brit. Columbia and World Wildlife Fund, pp: 114.

Lourie, S.A., A.C.J. Vincent and H.J. Hall, 1999. Seahorses: An identification guide to the world´s species and their conservation. Project Seahorse, London, UK, pp: 212.

Lucas, A., 1996. Bioenergetics of Aquatic Animals. Taylor Francis, G.B., pp: 169.

Lyytikäinen, T. and M. Jobling, 1998. The effect of temperature fluctuations on oxygen consumption and ammonia excretion of underyearling Lake Inari Arctic charr. J. Fish Biol., 52: 1186-1198.

Mallekh, R. and J.P. Lagardère, 2002. Effect of temperature and dissolved oxygen concentration on the metabolic rate of the turbot and the relationship between metabolic scope and feeding demand. J. Fish Biol., 60: 1105-1115.

Marais, J.F.K., 1978. Routine oxygen consumption of Mugil cephalus, Liza dumerii and L. richardsoni at different temperatures and salinities. Mar. Biol., 50: 9-16.

Masonjones, H.D., 2001. The effect of social context and reproductive status on the metabolic rates of dwarf seahorses (Hippocampus zosterae). Comparative Biochem. and Physiol. Part A, 129: 541-555.

Miller, D. and R.N. Lea, 1972. Guide to the Coastal Marine Fishes of California. Fish. Bulletin 157. State of California The Resources Agency Department of Fish and Game, pp: 249.

Navarro, J.C., F. Amat and J.R. Sargent, 1991. A study of the variations of lipid levels, lipid class composition and fatty acids composition of total lipids in the first stages of the life history of *Artemia* sp. Marine Biol., 11: 461-465.

Panigrahi, K., A.K. Panigrahi and B.N. Misra, 1984. Relationship of body length, body weight and oxygen uptake of Anabas scandens (Cuv. and Val.) during aging. J. Environ. Biol., 5: 9-13.

Payne, M.F. and R.J. Rippingale, 2000. Rearing West Australian seahorse, Hippocampus subelongatus, juveniles on copepod nauplii and enriched Artemia. Aquaculture, 188: 353-361.

Petit, G.D., 1973. Effects of dissolved oxygen on survival and behavior of selected fishes of western Lake Erie. Bull. Ohio Biol. Sur., 4:1-76.

Project Seahorse, 1999. http://www.seahorse.mcgill.ca

Rodier, J., 1981. Analisis de las Aguas. Omega, Barcelona, pp: 1059.

Sargent, J.R., L.A McEvoy and J.G. Bell, 1997. Requeriments, presentation and sources of polyunsaturated fatty acids in marine fish larval feeds. Aquaculture, 155: 117-127.

Schurmann, H. and J.F. Steffensen, 1997. Effect of temperature, hypoxia and activity on the metabolism of juvenile Atlantic cod. J. Fish Biol., 50: 1166-1180.

Shapawi, R. and G.J. Purser, 2003. The Value of Enriched Artemia in Supporting Growth and Survival of Juvenile Pot-Bellied Seahorses Hippocampus abdominalis. J. World Aquacult. Soc., 34: 533-541.

Tchernia, P., 1980. Descriptive regional oceanography. Pergamon Marine Series, New York, pp: 253.

Thurston, R.V. and P.C. Gehrke, 1993. Respiratory Oxygen Requirements of Fishes: Description of OXYREF, a Data File Based on Test Results Reported in the Published Literature. In Russo, R.C. and R.V. Thurston, (Eds.). Fish Physiology, Toxicology and Water Quality Management. Proceedings of an International Symposium, Sacramento, California, USA. US Environmental Protection Agency EPA/600/R-93/157, pp: 95-108.

Vincent, A., I. Ahnesjö, A. Berglund and G. Rosenqvist, 1992. Pipefishes and seahorses: Are they all sex role reversed. Trends. Ecol. Evolut., 7: 237-240.

Vincent, A.C.J., 1996. The International Trade in Seahorses. TRAFFIC Int. Cambridge UK, pp: 170.

Walsh, P.J., Y. Wang and C.E. Campell, 2001. Patterns of nitrogenous waste excretion and gill urea transporter mRNA expression in several species of marine fish. Marine Biol., 139: 839-844.

Wong, J.M. and J.A.H. Benzie, 2003. The effects of temperature, Artemia enrichment, stocking density and light on the growth of juvenile seahorses, *Hippocampus* whitei (Bleeker, 1855), from Australia. Aquaculture, 228: 107-121.

Wood, C.M., 1993. Ammonia and Urea Metabolism and Excretion. In: Evans, D.H. (Ed.). Physiology of Fishes. CRC Press, Boca Raton, FL., pp: 379-425.

Woods, C.M.C. and F. Valentino, 2003. Frozen mysids as an alternative to live Artemia in culturing seahorses Hippocampus abdominalis. Aquacult. Res., 34: 757-763.

Woods, C.M.C., 2002. Natural diet of the seahorse Hippocampus abdominalis. New Zealand J. Marine Freshwater Res., 32: 655-660.

Woods, C.M.C., 2003. Growth and survival of juvenile seahorse Hippocampus abdominalis reared on live, frozen and artificial foods. Aquaculture, 22: 287-298.

Yager, T.K. and R.C. Summerfelt, 1994. Effects of Feeding Frecuency on Metabolism of Juvenile Wallace. Aquacul. Eng., 13: 257-282.