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THE IMPORTANCE OF FISH NEUROHORMONAL RESPONSE REARED UNDER RECIRCULATING WATER SYSTEMS: A REVIEW

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ABSTRACT Fish farming prospects depend on the type of production system and the level of management. Therefore, the level of fish homeostasis and stress are important factors during their rearing period. Fish receive environmental stimuli mainly by their body receptors and react to them by an infinite number of outcomes influenced by various neurohormonal-biochemical processes. Using Recirculating Water Systems (RWSs), a notable reduction of undesirable hormonal and metabolic-biochemical functions could be easily achieved by preserving a proper combination of rearing water characteristics with tank size, fish rearing density, feeding practice and fish living ethology demands. These results could be further enhanced by combining the above mentioned parameters with rearing tank color and certain lighting conditions (photoperiod, spectrum, intensity). Also, by transmission of classical music into the fish rearing tanks, RWS facilities and proper rearing management can considerably contribute towards enhancing most fish anti-stress neurohormonal functions. That means that fish can feel closer to the way they should by living in their natural environment, without, however, facing its difficulties (e.g. enemies, water pollution, climate change). So, RWSs can provide for fish an excellent artificial "natural" living environment, while their construction and operation costs could be remarkably reduced not only by their low water requirements but also by using proper material, renewable energy resources and integration with aquaponics practices. Under these rearing conditions fish, being almost "happy", can show the highest possible levels of growth rate, final product quality and welfare, ensuring the farmers and consumers satisfaction as well.

Keywords: Fish neurohormonal response, Recirculated water systems application, Fish farming.

INTRODUCTION Generally, fish farming prospects should be focused on the achievement of maximum production of high quality and low cost marketable size fish, mainly for human consumption, in the shortest possible time, without causing environmental disturbance, especially to the aquatic environment (Papoutsoglou, 1991).
According to the experience gained since their first application, fish farming production systems could be classified into two main groups; the extensive and the intensive. This classification has been based on the level of human involvement in terms of using external origin feeds, rearing constructions and water manipulation, fish health care and farm operation management.

In the first group the extensive, the simple semi-extensive, the semi-extensive and the semi-intensive systems are included, while the second group is comprised by the intensive and the super-intensive systems.

In the extensive system fish feeding relies solely on the natural environment, while fish are caught by man-made traps. In the simple semi-extensive system the human implication is expressed by artificially increasing the natural productivity of the soil-water environment of a man-made earth pond; fish are continuously consuming only natural origin food. The semi-extensive production system involves the use of man-made earth ponds while fish, in addition to the natural food, are periodically offered supplementary feeds, mostly manufactured. In the application of the semi-intensive production system fish, besides their natural food consumption, are continuously offered supplementary feeds.

Using the intensive production system the rearing area has to be constructed by any suitable material except soil and fish feeding demands (live food or feeds) are met exclusively by man. The use of land based flow-through tanks or raceways and floating or not net cages are the constructive key points of this system. The super-intensive production systems are further characterized by an essential indoor operation and the potential for total control of rearing environment and fish behavior and physiology. In more details, correct water reuse manipulation, constructive material selection and covering the highest possible level of any particular fish species living ethology demands are the distinctive properties of these exclusively land-based systems (Papoutsoglou, 1996a, 2004a, Lekang, 2007, Craig and Hargreaves, 2008). They are the so-called Recirculating Water Systems (RWSs) or closed water systems or semi-closed water systems or Recirculating Aquaculture Systems (Fig. 1).

RWSs-REARING ENVIRONMENT-FISH PHYSIOLOGY Considering the attainment of their desirable homeostasis and stress levels, during their rearing period, fish must respond to the continuous combined effect of internal-external origin stimuli with the minimum energy expenses, by the most simple expression of all biochemical functions determined by their species-specific physiology.

Defining stress resembles a scientific or philological or etymological or terminological adventure, aiming to provide a correct description of all physiological functions illustrating the reactions or responses of organisms caused by several stimuli of internal or external origin, which could be called stressors. The difficulties encountered are related to the fact that a universally accepted definition of stress should be suited to or connected with or associated with experienced or not, expected or not, disturbances of homeostasis, resulting in or being caused by stimulation of specific neurohormonal-enzyme functions, usually affiliated with energy expenses or even causing death. However, regardless of precise definition of stress, its practical meaning in finfish farming has been linked to and been realized through financial losses that may originate either from acute and/or chronic stress, the latter being the most common and important in ongrowing finfish rearing periods (Barton, 1997, Papoutsoglou, 1998, 2005).

According to present knowledge fish can “communicate„ with their living environment through the response of their senses, using their external body surface as a major stimuli receptor. However, fish can achieve the highest level of understanding their living conditions by using their mouth cavity, eyes, nostrils, barbels, gills, fins, lateral line, internal ears, body temperature receptors and digestive tract. In almost all cases, stimuli after their reception are forwarded to the brain from where fish responses start and are

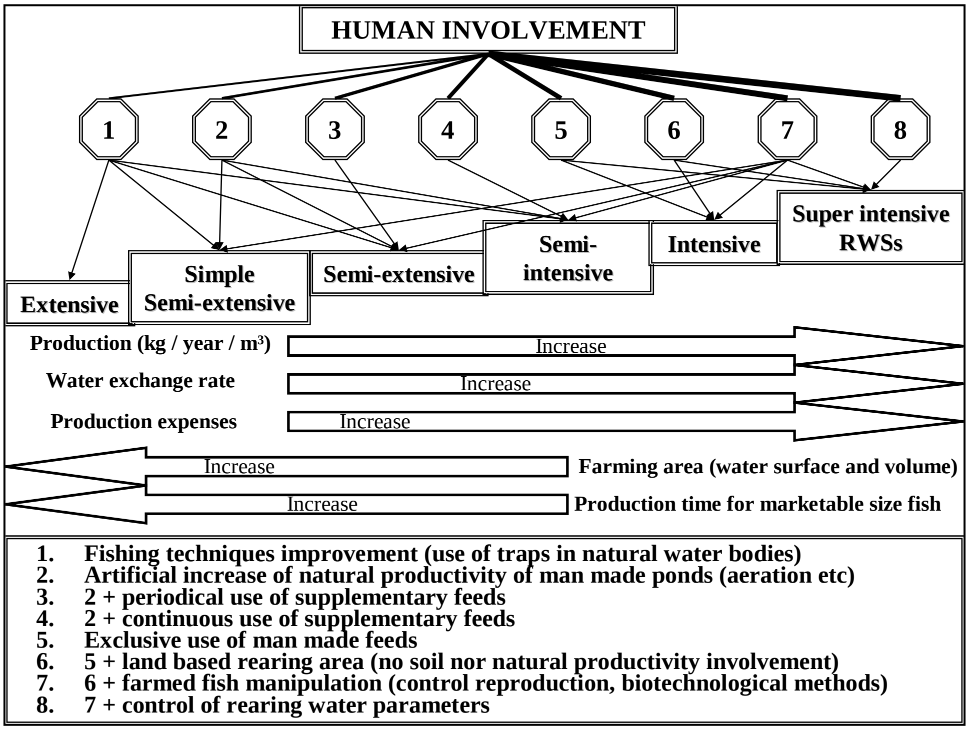

Figure 1. Simplified presentation of fish production systems characteristics according to human involvement (based on Papoutsoglou, 1994).

sent to their targets. This is realized by means of the outcome of an infinite number of various interactions of several neurohormonal and biochemical processes (Papoutsoglou, 1998, Collin and Marshall, 2003, Bernier et al., 2009, Wells, 2009).
Every response of farmed fish to the environmental stimuli is related to the combined effect of rearing water parameters, construction-facilities characteristics and rearing condition-management. Fish, being aquatic and poikilothermic animals and able to increase their body dimensions, as long as they live, are continuously trying to maintain their osmotic and ionic balance and proper food utilization to cover all their physiology-metabolic demands with the minimum energy cost.

The main rearing water parameters are its chemical composition, as well as, its physical and hydrological characteristics.

The effects of rearing water suspended matter on fish neurohormonal status are depended on their concentration level, chemical origin (organic or not) and duration of their presence, in relation to fish species, age-biological stage and physiological status. Provoking the excretion of excess mucus by gills cells, suspended matter, usually cause reduced oxygen intake and hypoxia symptoms. These are leading to suspension of growth hormone excretion and balance disorder of thyroid gland hormones (Hughes, 1981, Adams, 1990, Papoutsoglou and Tziha, 1994, McMaster et al., 1995, Karakatsouli et al., 2006a). The main neurohormonal axes involved are gills and/or nostrils buds and/or taste buds and/or optic nerves→ brain centers → hypothalamus → hypophysis → adrenal system by which cortisol and haematocrit levels are increased. By RWSs use stress-induced energy demands could be minimized in farmed fish since high particulate matter levels could be readily removed from rearing water by properly designed and operated mechanical filters.

Gills are the main fish reception site of the fluctuation of water ions (Na^+, K^+, Cl^-, Ca^{++}, Mg^{++} etc), gases (O_2, CO_2), several chemical compounds (NH^+_4, NH_3 etc) and pH levels. Chloride cells of gill epithelium response to the water ion concentration requires energy as well as the activation of the neurohormonal axis hypothalamus hypophysis → chromaffin tissue followed by corticosteroid hormones (cortisol, cortisone) and catecholamines (adrenalin, noradrenalin, dopamine) excretion. These hormones provoke the activation of pancreatic islets and thyroid gland by which blood glucose is increased and energy demands, mostly caused by Na^+ and K^+ pumps, are satisfied. However, depending on fish age-biological stage and species (sea or fresh water), apart from the above mentioned, several other tissue (brain, heart, kidney, the corpuscles of Stannius, ultimobranchial gland, gonads, intestinal epithelium) hormones and biochemical axes could be involved. Their aim is to achieve fish growth and reproduction by a continuous ionic and osmotic homeostasis. Among others, growth hormone, angiotensins, VIP, insulin, calcitonin, stanniocalcin, IGFs, natriuretic peptides, prolactin, gonadal hormones could be mentioned (Freeman and Sangalang, 1985, Papoutsoglou and Abel, 1988, Tomasso, 1994, Jobling, 1995, Papoutsoglou, 1996b, Papoutsoglou and Tziha, 1996, MacIntyre et al., 2008). It is clear therefore that rearing water chemistry should not cause any extra energy cost by unnecessary involvement of farmed fish neurohormonal axes during the farming period. This target could be only obtained using RWSs by which a relatively moderate control of the levels of the most important water chemistry parameters could be achieved. Among them salinity, oxygen, unionized (toxic) ammonia, chloride, pH and colloid substances should be mentioned. While almost all other parameters could be manipulated and monitored by instrumental techniques, the most common method for toxic ammonia removal is associated with accurate function of the so called biological filters (nitrification filters, biofilters), using special substrates for exclusive bacteria (*Nitrosomonas, Nitrobacter*) populations establishment.

The special importance of the effects of rearing water temperature on fish physiology is related to the outcome of the interactions between species living ethology (warm or cold water), size, biological stage and sex, neurohormonal status, as well as several other rearing parameters. Among them water chemical composition and circulation, photoperiod, rearing density and feeding practice could be mentioned. The main hormonal tissues involved are hypophysis, gonads, thyroid gland, pancreatic islets, adrenal tissue and digestive tract. Generally, fish metabolic-enzymatic functions are strongly influenced by notable water temperature fluctuations leading either to positive or negative results concerning mainly fish growth rate, ionic-osmotic homeostasis and sensitivity of their immune system. This is because within fish water temperature survival range there are three distinct points strongly related to their physiology level. At the highest point fish are spending more metabolic energy than that they consume. In this case fish are not increasing their body weight and their homeostasis is not easily achieved. At about a middle point fish are gaining body weight since the consumed food energy is more than that required to cover their basic metabolism energy demands, providing that water oxygen level is corresponding to temperature. At a lower than the previous point, fish growth rate attains its lowest level, mainly because of the notable decrease of fish appetite related to minimized energy demands caused by the low water temperature. It is obvious therefore, that fish physiology of nutritional status could be manipulated as long as water temperature could be technically handled (Papoutsoglou and Papaparaskeva-Papoutsoglou, 1978a, b, Elliot, 1981, Papoutsoglou, 2009). This possibility could only be obtained by RWSs application, which however may represent, besides the initial capital, the highest operation cost especially when conventional energy sources are used. Nevertheless, by using renewable energy resources (solar, geothermal), or integrated production systems (e.g. fish rearing tanks inside green houses), the cost of water temperature control could reach its lowest level (Papoutsoglou, 2004b).

Serious consequences on fish metabolic-physiology conditions could be derived when they have to face increased rearing water velocity and/or continuous turbulence. Rearing water hydrology characteristics within the rearing tanks, is expressing the outcome of the interaction between water inlet and outlet, as well as, tank size and shape (e.g. elongated or round). This should be leading to the establishment of such water circulation conditions that will permit fish to express their physiology-derived swimming action only. Under unfavorable circumstances there is a fish anti-stress neurohormonal-enzymatic axis activation caused by the excess energy demands, which usually is associated with decrease of fish growth rate and increase of production cost (Jobling, 1995, Papoutsoglou 2004a). By suitable regulation of all water motion characteristics within RWSs rearing tanks, neither extra energy cost nor fish homeostasis disturbance should be expected.

Furthermore, fish rearing density could be related to their homeostasis level, influencing their stress status by means of feeding performance, quality of final products and growth rate. The choice of the proper rearing density should be mainly based on fish ethology of living in relation with their feeding ethology, nutrition type, biological stage and feeding practice. When fish rearing density is higher than it should be, fish population response may result in a progressive increase of their size heterogeneity, aggressiveness, body shape deformities and establishment of dark coloration. Fish anti-stress response to the complexity of these interactions causes an excess of energy demands, which are not covered to full extent by the food amount a number of specimens could consume. Also, rearing density being associated with fish living ethology should provide them the possibility to express their natural swimming activities or to lie on the bed of the rearing tank (in case of flatfish) the way they instinctively “know„ by their nature. In other words, rearing density should be combined with rearing tank size, shape and available water volume, in order to achieve the least possible (if at all) fish stress level. In addition, issues of fish physiology are increasingly complicated when more than one fish species have to be reared in the same tank. The more the similarities, mostly in terms of living and feeding ethology demands, among the commonly reared fish species exist, the more the chances for success of such trials are, especially when RWSs are applied (Papoutsoglou et al., 1979, 1987, 1992, 2006, Karakatsouli et al., 2006b).

Farmed fish feeding practice consists of food chemical composition (in terms of energy and nutrient contents) and texture, feeding rate level and daily amount of food needed, number of daily meals and mode of feed supply to the fish (by hand or not). Considering that farmed fish cannot be fed individually, the success of feeding practice is almost entirely depended on farmer knowledge and management skills. It is obvious that no matter how carefully food could be provided to a farmed fish population, it is almost impossible to equally allocate feed to every specimen in a daily meal. This is mostly due to the genome differences among individuals in association with the effect of hierarchies, which could be developed within farmed populations. In addition, these differences could be depended on fish species living ethology, as well as, on the specimen size differences which usually occur some time after the beginning of the rearing period. Thus, feeding practice ought to alleviate as much as possible the consequences of fish stress level, which is inevitable under domesticated living conditions, mostly by successful management of the aggressive behavior and by giving the chance even to the smallest individuals to access food. Food amount and chemical composition are the most critical factors for adequately satisfying fish energy and nutrients demands, for maintaining their homeostasis and obtaining their highest potentially growth rate. Therefore, at least the basic nutritional demands of farmed fish concerning their living ethology (fresh or sea water, stenohaline or euryhaline) as well as their neurohormonal-biochemical aptitude and presupposition to synthesize polyunsaturated fatty acids must be known as much as possible. It has to be emphasized that all fish species should be nutritionally provided by all necessary quantities of ions and molecules in order to keep all their tissue cell membranes capable to function properly, in terms of their plasticity and permeability. Insulin, catecholamines, cortisol, ACTH, TSH, MSH and growth hormone are the main hormones the interaction of which, depending on fish nutritional status, support critically the existence of their homeostasis and normal growth (Papoutsoglou, 1997, 2002, Papoutsoglou and Papaparaskeva-Papoutsoglou, 1978b, Papoutsoglou and Voutsinos, 1988, Aragão et al., 2008). Therefore, it must be addressed that the greatest possible level of the endocrinological regulation of fish feeding-nutrition could be obtained under satisfactorily equipped and managed RWSs.

Generally, fish living ethology demands vary among species, as well as in relation to the biological stages of each species. Apart from the defined involvement of all biotic factors, the existence of proper combination of all abiotic factors, which could make up the living environment of each farmed fish species during all their biological stages, is almost equally important. Thus, the proper combination of all water parameters and feeding practice with the quality and technical-operative characteristics of all the facilities used, seems to be the only promising way to cover successfully all fish physiology demands. This, with the less possible stress-induced energy expenses, could be achieved, especially when RWSs are used (Papoutsoglou, 2005).

The color of fish living environment during the rearing period is the constant final outcome of the interactions between water color (depending mostly on suspended and dissolved matter), lighting conditions (photoperiod, spectrum, intensity), tank color and fish loading level. According to present experience worldwide, the rearing environment color could be considered as one of the major rearing parameters influencing farmed fish growth rate. This is because fish response could be related to defense behavior, (intending to adapt accordingly body color), part of reproduction performance and stressful rearing conditions of external or internal origin or both. The involved neurohormonal axes eyes and/or pineal organ → hypothalamus → hypophysis control the excretion and distribution of specific pigment granules (mainly melanin), as well as the number of chromatophore cells of fish skin by the implication of aMSH, MCH, catecholamines (e.g. adrenaline) and cortisol. The final results of fish biochemical responses mentioned above are always depended on the specific interactions between biotic and abiotic parameters. In a number of fish species studied it has been proven that skin darkening is associated with enhanced release of aMSH caused by black background, while white background has been related to skin lightening due to increased levels of MCH excretion. Generally, fish living under chronic stressful background color conditions, which could be associated with different growth performances and immune system status, are expressing different energy adaptation expenses depended on their plasma cortisol and glucose level released, as well as different liver (e.g. total lipid content) and brain (e.g. serotonergic and dopaminergic activity) physiology. Also, it should be emphasized that the final effect of lighting conditions could be easily modified according to the specific combination applied among photoperiod, spectrum and intensity levels. These modifications are definitely connected with various fish energy cost levels influencing their physiology and growth (Papoutsoglou, 2001, Papoutsoglou et al. 2000, 2005, Karakatsouli et al., 2007a,b, 2008, Martinez-Cardenas and Purser, 2007). So, by a sufficient monitoring of suitable lighting devices, farmed fish could be offered the chance to enjoy, from that point of view, a real anti-stress rearing environment. It is obvious that the establishment of such appropriate rearing conditions could only be performed by RWS application.

It is well known that a farmed fish population is not able to avoid exposure to a considerable extent of waterborne and airborne noise-sound. Generally, depending on specific circumstances, the more the production system is intensified the more the waterborne noise could be greater due to absolutely indispensable human involvement. Such kinds of conditions have to be particularly accepted when specific machinery must be used. This is the case of RWSs whose application is seriously depended on special apparatus proper operation. Electrical generators, biological and mechanical filters, pumps (water and air) as well as, blowers, are included, most of which usually function quite close to farmed fish populations, if not even inside rearing tanks. According to well documented results, fish being exposed to ambient noise-sound could be stressed to the point of disturbance of their homeostasis, which is usually expressed by immune system weakness, change of nutritional physiology functions and reduction of growth rate. However, the final outcome of sound effects on farmed fish physiology is depended on the level of many variables. Among them, as the most important to be mentioned, are species-specific physiology, in terms of fish size-biological stage, living ethology and especially hearing abilities, in relation to chronic level of exposure to different sound levels and frequencies, as well as, fish acclimated behavior. Also, it should be emphasized that sound effects could be modified according to other rearing parameter involvement and especially to fish nutritional status and lighting conditions applied. The origin of fish response to sound stimuli, by means of the outcome of many biochemical pathways involving hormones, enzymes and neurotransmitters, is located on their auditory system, which is composed by inner ear, swim bladder, lateral line and brain anatomy or more "practically„ by all their body surface. Fish brain anatomy and function and its interaction with autonomic and blood circulation systems define the physiology status of liver, spleen, kidneys, as well as digestive enzymes activity and finally growth rate perspectives. Thus, fish can, at a given time, state the characteristics of the consequences that sound may cause to them. These could be either harmful or not, depending on the level of stress or anti-stress effect that sound might be associated with. For example, common farmed fish species response to transmission of classical music pieces (e.g. Mozart) demonstrated that it could react as a stress release factor improving homeostasis status and growth rate. Music transmission implementation could be easily and practically a part of the rearing components of all fish production systems (except the extensive one). However, it is only the RWS application that gives the opportunity to farmed fish to express their satisfaction and pleasure living in an almost complete anti-stress environment by the outcome of their neurohormonal responses, including those of music stimuli (Fay and Popper, 1999, Bart et al., 2001, Papoutsoglou and Lyndon, 2005, 2006a, b; Papoutsoglou et al., 2007, 2008, 2009, Simmons and MacLennan, 2007, Davidson et al., 2009).

CONCLUSION Although the number of RWSs designs is endless, there are only two equally important, fundamental principles that must be seriously considered as long as

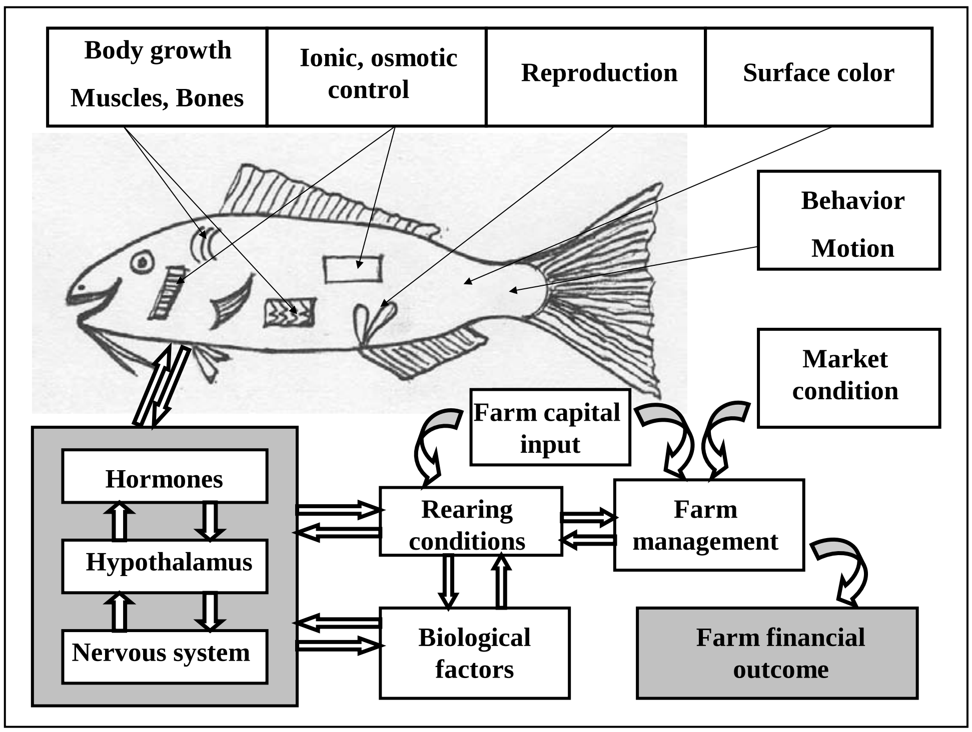

Figure 2. Simplified presentation of farmed fish neurohormonal system involvement in farms financial outcome (based on Papoutsoglou, 1998).

fish farming must be operated as a business. The first one is associated not only with the correct choice of the most necessary components but also with the material that they have to be made of, as well as, with the level of the simplicity they could be operated. The second is related to the quality of the management of both the components operation and fish living conditions (Wheaton, 2008). As long as fish homeostasis should be the first priority, proper management intention must continuously be focused on keeping the rearing water quality at the highest possible level by means of its chemical, biological and hydrological parameters. A properly equipped RWS should be characterized by the presence of mechanical filters for suspended matter removal, biological filters for toxic ammonia oxidation and chemical filters devices for colloid substances removal. Also, by

UV lamps for pathogen elimination, aerators for air and/or pure oxygen supply, rearing water temperature control facilities, artificial lighting, air-oxygen and water pipes and pumps, electrical generators and alarm systems. The first step of a desirable management quality is related to the manner of the arrangement of the RWSs components in a given size and shape area, while it should never stop to provide in the best way a continuous proper fish and facilities attention. Thus, constructions operation and conditions should be checked continuously, while water parameters and fish physiology characteristics must be determined at regular-safe intervals. In addition, farmers should improve their managing skills by every day experience expressing their true will about what they are doing, attending closely not only fish rearing process but also being continuously aware of market conditions.

Low water and land requirements, independence from weather conditions, integration with aquaponic techniques, protection of the aquatic environment from outlet discharge, use of renewable energy resources and avoidance of the consequences of climatic changes are the main unquestionable positive characteristics of RWSs application. While, by ensuring the highest fish growth and quality, the most profitable food conversion rate, the highest rearing density and final annual production of mono-or polyculture (fish number or biomass/m^3 of rearing water), as well as the minimum risk of infectious disease outbreak, RWSs can counterbalance the risks involved and the high capital and operation investment needed. Moreover, it should be emphasized that by RWSs use not only rearing water but also all rearing environmental parameters could be controlled according to farmed fish species neurohormonal demands. In other words it is the only fish farming system by which the financial outcome of a farm could be continuously improved based on an uninterrupted proper utilization of fish specific neurohormonal response, according to their rearing conditions (Fig. 2). This means that RWSs can provide the farmed fish with an excellent artificial “natural„ living environment. Although the number of the interdependent questions to be answered seems to be endless, at the moment, the application of RWSs is the only way for fish and man to enjoy a real “communication”. This assertion is based on the certainty that fish not only hear but also they can “listen„ to the environmental stimuli, although they lack brain cortex. Under these rearing conditions fish could be able to return human love and care by expressing their “happiness„ and welfare and ensuring the farmers and consumers satisfaction as well (Papoutsoglou, 2000, Conte, 2004, Brown et al., 2008).

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APPENDIX A

Abbreviations: IGFs = Insuline like Growth Factors; VIP = Vasoactive Intestinal Peptide; ACTH = Adreno Cortico Tropic Hormone; TSH = Thyroid Stimulating Hormone; MSH = Melanocyte Stimulating Hormone; MCH = Melanine Concentrating Hormone; UV = Ultra Violet.