EastMed TECHNICAL DOCUMENTS 10

THE PUFFER FISH
LAGOCEPHALUS SCELERATUS (GMELIN, 1789)
IN THE EASTERN MEDITERRANEAN

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

eastMed

The Puffer Fish *Lagocephalus sceleratus* (Gmelin, 1789) in the Eastern Mediterranean

Prepared by

Manal R. Nader

Shadi Indary

Layal Ephrem Boustany

ITALIAN MINISTRY OF AGRICULTURE, FOOD AND FORESTRY POLICIES

Hellenic Ministry of Foreign Affairs

Hellenic Ministry of Rural Development and Food

GCP/INT/041/EC – GRE – ITA

Athens (Greece), March 2012

The conclusions and recommendations given in this and in other documents in the *Scientific and Institutional Cooperation to Support Responsible Fisheries in the Eastern Mediterranean* series are those considered appropriate at the time of preparation. They may be modified in the light of further knowledge gained in subsequent stages of the Project. The designations employed and the presentation of material in this publication do not imply the expression of any opinion on the part of FAO or donors concerning the legal status of any country, territory, city or area, or concerning the determination of its frontiers or boundaries.

Preface

The Project "Scientific and Institutional Cooperation to Support Responsible Fisheries in the Eastern Mediterranean – EastMed" is executed by the Food and Agriculture Organization of the United Nations (FAO) and funded by Greece, Italy and EC.

The Eastern Mediterranean countries have for long lacked a cooperation framework as created for other areas of the Mediterranean, namely the FAO sub-regional projects AdriaMed, MedSudMed, CopeMed II and ArtFiMed. This made it more difficult for some countries in the region to participate fully in international and regional initiatives for cooperation on fishery research and management. Following the very encouraging experience of technical and institutional assistance provided to countries by the other FAO sub-regional Projects,

EastMed

was born to support the development of regional cooperation and the further development of multidisciplinary expertise, necessary to formulate appropriate management measures under the FAO *Code of Conduct for Responsible Fisheries* and the principles of the *Ecosystem Approach to Fisheries (EAF)* to ensure rational, responsible and participative fisheries management

The project's **longer-term objective** aims at contributing to the sustainable management of marine fisheries in the Eastern Mediterranean, and, thereby, at supporting national economies and protecting the livelihoods of those involved in the fisheries sector.

The project's **immediate objective** is to support and improve the capacity of national fishery departments in the sub-region, to increase their scientific and technical information base for fisheries management and to develop coordinated and participative fisheries management plans in the Eastern Mediterranean sub-region.

FAO – EastMed Project HQ
Androu 1,112 57 Athens
Greece
Tel: +30210 8847960
Fax +30210 8837600
E-mail: Eastmed@fao.org
URL://www.faoeastmed.org

Publications

EastMed publications are issued as series of Technical Documents (GCP/INT/041/EC – GRE – ITA/TD-00) and Occasional Papers (GCP/INT/041/EC – GRE – ITA/OP-00) related to meetings, missions and research organized by or conducted within the framework of the Project.

Occasionally, relevant documents may be translated into national languages as EastMed Translations (GCP/INT/041/EC – GRE – ITA/ET-00)

Comments on this document would be welcomed and should be sent to the Project headquarters:

FAO – EastMed Project
Androu 1,112 57 Athens
Greece
Eastmed@fao.org

For bibliographic purposes this document should be cited as follows:

Nader M., Indary S., Boustany L., 2012. FAO EastMed The Puffer Fish *Lagocephalus sceleratus* (Gmelin, 1789) in the Eastern Mediterranean. GCP/INT/041/EC – GRE – ITA/TD-10

Preparation of this document

This document is the final version of the FAO EastMed The Puffer Fish *Lagocephalus sceleratus* (Gmelin, 1789) in the Eastern Mediterranean compiled by the FAO-EastMed Project (Scientific and Institutional Cooperation to Support Responsible Fisheries in the Eastern Mediterranean).

Acknowledgements

The authors would like thank Dr. Hatem Hanafy Mahmoud, University of Alexandria, Alexandria, Egypt; Dr. Panagiota Peristeraki, Hellenic Center for Marine Research, Herklion, Greece; Dr. Stefanos Kalogiru, Hellenic Center for Marine Research, Rohdes, Greece; and Dr. Maria Corsini-Foka, Hellenic Center for Marine Research, Rhodes, Greece for the valuable information they provided for the compilation of this review.

Special acknowledgement is extended to the Department of Fisheries and Wildlife of the Ministry of Agriculture in Lebanon and the FAO Representation in Lebanon for their essential logistical and technical assistance.

Nader M., Indary S., Boustany L., 2012. FAO EastMed The Puffer Fish *Lagocephalus sceleratus* (Gmelin, 1789) in the Eastern Mediterranean. GCP/INT/041/EC – GRE – ITA/TD-10. Athens 2012: 39 pp.

ABSTRACT

In the Mediterranean region, the fisheries sector has been suffering from the impacts of migration of aquatic organisms through the Suez Canal. Among the most devastating species to both fisheries and habitats is the aggressive predatory puffer fish *Lagocephalus sceleratus*. The fish bio-concentrates Tetrodotoxin (TTX), a very potent poison making the fish unmarketable and poses a great risk to human health if consumed. In addition, *L. sceleratus* has been recorded to destroy fishing nets and lines leading to economic losses for fishers. The objective of this review is to describe the status of *L. sceleratus* and its commercial applications, if any, around the world with emphasis on the Mediterranean Sea. Given the lethal TTX toxicity of *L. sceleratus*, and the strict regulations passed around the world preventing its fishing and consumption, coupled with the little knowledge available about its biology and its bio-concentration of TTX, it is not recommended at this stage to consider marketing the fish to consumers. Even-though the aquarium and capture-based aquaculture industries may provide partial solutions, the best option lies in creating, and in association with pharmaceutical companies, multidisciplinary laboratories in Mediterranean countries with the main objective of isolating TTX from puffer fishes including *L. sceleratus* and investigating the toxin's potential use in the pharmaceutical industry. Such an option would create many employment opportunities in the region, but more importantly, it will create a fishery that will yield economic benefits to the fishers and control wild populations through increased fishing pressure. However, such an initiative requires many investigative activities about the biology of the fish as well as very strict permitting and regulatory processes in the countries where such a fishery might be established.

Table of Contents

Table of Figures

THE PUFFER FISH

***LAGOCEPHALUS SCELERATUS* (GMELIN, 1789)**

IN THE EASTERN MEDITERRANEAN

1. Introduction

Increasingly, the breaking by humans of natural barriers is leading to the spreading of species into ecosystems to which they are alien. This dispersal can impact native species and the equilibrium of both terrestrial and marine invaded ecosystems, leading to evolutionary pathways which are not yet fully understood, (Golani 2011). Much attention is being given to the problem today since these introductions are one of the most significant threats to ecosystem biodiversity, structure, and function (Oral 2010). Evidence shows that invasions lead to a decline in the number of native species, disturbance of certain valuable processes, economic losses, and to the introduction of diseases and pathogens. In certain extreme cases, native species can become extinct due to competition with or predation by the invasive organisms (Oral 2010). Frequently, invasive species are discovered after they have already been in the new habitat for an extended period of time making it difficult to determine how the invasion started and when. Apart from this, a species may fail to establish a viable population in a certain habitat, and therefore the invasion might never be accounted for (Golani 2010). Lately, the problem of climate change further complicated the subject of alien species by its uncertain impact on species distribution, abundance and behavior (Argüelles 2008; Burgiel 2010).Cases in which species have migrated due to changing surroundings have already been recorded and correlated to human induced climate change (IUCN 2000).

The Mediterranean Sea is home to numerous invasive marine species where some of them have already established prosperous populations. These invasives might have entered through several routes: the Suez Canal, the Gibraltar Strait, ship ballast water and accidental release, among others. Nevertheless, the Suez Canal is considered as the major route of migrating indo-pacific species, also referred to as Lessepsian species, from the Red Sea into the Mediterranean. The opening of these two different seas to each other, resulted in very significant consequences which were not accounted for when the canal was built. Marine organisms migrated from the Red Sea into the Mediterranean in relatively large numbers, although migrations do occur in the opposite direction but on a lower scale. Nonetheless, the Gibraltar Strait, ship`s ballast water and accidental release remain an important source for the introduction of alien species in the Mediterranean. In total, the number of alien species has reached more than 790 in the Mediterranean Sea, with some of the invaders through the Suez Canal having been very successful colonizers of Mediterranean marine ecosystems (Galil & Zenetos 2002; Zenetos 2005; Golani & Azzurro 2007; Golani 2010; Oral 2010).

The Suez Canal between the Mediterranean and Red Seas (Figure 1) is recognized by far to be the foremost path for invasive species, it was built in 1869 in order to facilitate the trade between Europe and the Far East by the engineer Ferdinand de Lesseps, after whom the species that migrated to the Mediterranean were named. The canal, connecting these two hydrographically and biologically dissimilar water bodies, extends 162.5 km and is relatively narrow and shallow for most of its length. In view of the fact that sea surface temperatures have been rising in the last twenty years, it has been suggested that the Eastern Mediterranean conditions are becoming more and more suitable for the growth, reproduction, and survival of tropical species, offering the aliens various advantages when competing with native species (Golani 1998; IUCN 2008; Bernardi et al., 2010; Maiyza et al., 2010). Today, more than 300 Indo-Pacific species, including fish, macrophytes, and invertebrates, have entered the Eastern Mediterranean through the Suez Canal. The relatively shallow water of the Canal with an average depth of around 10 m is considered as a major physical barrier for the migration of deep water species. Accordingly, most of the invasive species can be found at depths of less than 70 m in the Eastern Mediterranean (Peristeraki 2006; Oral 2010; Torcu 2010).

Figure 1. Mediterranean Sea (Ozturk, B. 2010)

The study of the Lessepsian migration presents many advantages to scientists as the date of the opening of the Suez Canal is known as is the origin of the invaders. Therefore, extensive research has been, and is still being carried out to monitor the arrival of invasive species, study their impact on the marine ecosystem as a whole and to provide predictions. Given that it is predicted that invasions of species of the Mediterranean will continue and since changes in ecosystems as well as in coastal fisheries have already been reported, this becomes an ecological and economic issue which must be addressed and constantly studied and monitored (Bariche et al., 2004; Corsini-Foka 2005; Corsini-Foka 2006; IUCN 2008; Oral 2010).

Lagocephalus sceleratus is one of the Lessepsian species which has invaded the Eastern basin of the Mediterranean Sea. It was first recorded in Gokova Bay-Turkey in 2003, while the previous 1977 record by Mneimné was a misidentification of the similar puffer fish *Lagocephalus suezensis*. *L. sceleratus* has already established a population which is colonizing new territories of the Eastern Mediterranean at a relatively rapid rate. This rapid expansion can be easily observed as the fish had reached the Aegean Sea in 2006, three years after it was reported for the first time in Turkey in 2003 (Bilecenoglu et al., 2006; Kassapidis et al., 2007; Carpentieri et al., 2009; Pancucci-Papadopoulou et al., 2011). Today, it is regarded to be among the worst invasive species in the Mediterranean Sea with a significant impact on the surrounding ecosystem and on the fisheries sector (Zenetos et al., 2005; Peristeraki 2006; Streftaris & Zenetos 2006; Ozturk 2010). More importantly, *L. sceleratus* is considered to be a serious hazard to consumers since it contains a strong marine toxin called tetrodotoxin (TTX), which can be lethal to humans.

The purpose of this report is to review the biological and ecological characteristics of *L. sceleratus* with a focus on the potential commercial use of this species.

2. Characteristics of *Lagocephalus sceleratus*

2.1 Biology of *L. sceleratus*

Puffer fish, blow fish, balloon fish, toad fish, and globe fish, are all generic names given to several members of the fish family Tetrodontidae, which includes 187 different species worldwide (West 2009; Homaira 2008).

The German scientist, Johan Gmelin, first identified *L. sceleratus* of the family Tetrodontidae in 1789. Around the world, many different scientific names are used to refer to this species: *Fugusceleratus, Gastrophysis sceleratus, Gastrophysus sceleratus, Gastrophysus scleratus, Lagocephalus scleratus, Pleudranacanthus sceleratus, Pleuranacanthus sceleratus, Spheroides sceleratus, Sphoeroides sceleratus, Sphoeroides scleratus, Tetraodon bicolor, Tetraodon blochii, Tetraodon sceleratus, Tetrodon sceleratus* (FishBase 2004). For the purpose of this report, the scientific name *L. sceleratus* adopted by FishBase and by the CIESM Atlas of Exotic Species is used.

Commonly known as the Silver-cheeked toadfish, *L. sceleratus* is a widely distributed species which inhabits the tropical Indian and Pacific Oceans, from which it originates (Figure 2). Generally, this species inhabits sandy or muddy substrate areas near shallow coral reefs at depths reaching 100 m. However, it has been also found living at a depth of 250 m in the Red Sea (Yaglioglu 2011). *L.sceleratus* entered through the Suez Canal and rapidly invaded the Eastern Mediterranean Sea reaching the western part of the basin (Yaglioglu 2011). Sandy bottoms were found to be very important for the juveniles of this species, while the adults are more common in *Posidonia oceanica* meadows. Its relatively rapid spread throughout the Mediterranean Sea shows that this fish can adapt easily to new habitats (EastMed 2010b; Kalogirou 2011).

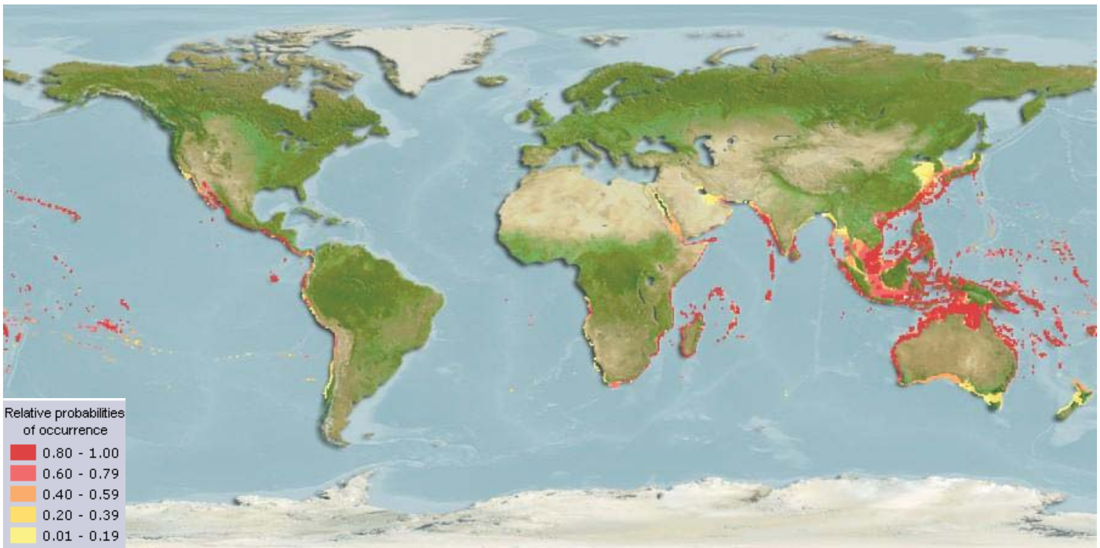

Figure 2. Distribution range of *L. sceleratus* (FishBase 2010)

The puffer fish *L. sceleratus* is one of the largest members of its family, reaching 110 cm and 7 kg. It has an elongated body slightly compressed at the sides and similarly to other puffer fishes, it is capable of inflating its body by swallowing water (Figure 3). This characteristic is common to puffer fishes, which are relatively slow swimmers and have therefore evolved this ability in order to appear larger to predators (Takeda 1996). Scales on the body are absent, while only very small spinules can be observed on its belly and on its dorsal surface. The lateral lines are easily visible and the body is dark brown with regular blotches on its back, except for the belly which is white (Bilecenoglu 2006). It has two strong teeth in each jaw which are capable of ripping and damaging fishing nets and longlines (Figure 4) giving the family of *L. sceleratus* its name (tetras= four; odontos= tooth). In the Mediterranean, this fish was found to be carnivorous, feeding mainly on shrimps, but also on crabs, fish including individuals of its own species, squids, and cuttlefish (EastMed 2010b; Aydin 2011). Studies revealed an ontogenetic diet that shifts to a mollusk diet with increasing size. This can be explained by the shift in habitat from sandy bottoms where invertebrates are its main preys, to *Posidonia oceanica* meadows where the main food items become mollusks such as *Sepia officinalis* and *Octopus vulgaris* (Kalogirou 2011). In addition, interviews with local fishermen along the Lebanese coast have revealed the opportunistic behavior of this species as it attacks fishes captured in the nets and lines and seriously damages fishing gears and catch. This behavior has also been observed in other countries such as Cyprus and Greece

(EastMed 2010b). Furthermore, this species accumulates Tetrodotoxin poison through its diet, which is a non-protein, organic compound, known to be one of the strongest marine toxins and being 1200 times more toxic than cyanide.

Figure 3. *L. sceleratus* (Kolpostrianta - Rhodes, 5-10-2007, Greece, by Stamatis Stamatellos)

Very high densities of this fish were caught during samplings in the Eastern Mediterranean and it was found to rank among the 10 most dominant species in terms of biomass (Streftaris & Zenetos 2006; Kalogirou 2010). *L. sceleratus* has been reported to have the ability to quickly spread and exploit resources, competing with the native carnivores. Therefore, the rapid expansion of this species can affect diversity as well as the abundance of native species in the near future, especially with changing environmental conditions through climate change since this is expected to smooth the progress of its adaptation to the new environment (Kalogirou 2010; Aydin 2011). When compared to the expansion pattern of other invasive species and taking into account that *L. sceleratus* was recorded for the first time in the Eastern Mediterranean in 2003, it can be concluded that it is one of the fastest expanding Lessepsian immigrants up to now (Peristeraki 2006).

Figure 4. Strong teeth of *L. sceleratus* (Daily Star 2011)

2.2 Tetrodotoxin

2.2.1 Origin

The production of Tetrodotoxin (TTX) by marine bacteria has been investigated since the 1980's, with studies focusing mainly on species belonging to the genus *Vibrio* and on a lower scale on bacteria of the genus *Pseudomonas*. *Vibrio* bacteria are a genus of aquatic, comma-shaped, very motile bacteria which belong to the family Vibrionaceae and which can cause many serious diseases to animals. The most common source of bacteria associated with TTX production is *Vibrio alginolyticus*. For instance, puffer fishes, Chaetognaths, and Nemerteans have been shown to contain *V. alginolyticus*. To confirm the bacteria's role in the production of TTX, many screening experiments have been done which revealed that 10 strains of the *Vibrio* family produce this toxin. TTX is taken up from the food chain, but the transfer, accumulation, and elimination mechanisms of TTX obtained via preys remain unclear.

In the past, TTX was believed to be found only in puffer fishes and it was controversial whether TTX was produced by the fish itself or was accumulated from the surrounding environment. Several findings, such as the distribution of TTX among other organisms, TTX production by certain marine bacteria, and the absence of TTX toxicity in artificially reared puffer fish with non-toxic diets later showed and proved that the toxin is exogenous. Although TTX has been isolated mainly in puffer fishes, various other species accumulate it, including parrot fishes, toads of the genus *Atelopus*, several species of blue-ringed octopuses of the genus *Hapalochlaena*, several starfish, an angelfish, a polyclad flatworm, several species of arrow worms, several ribbon worms, and many species of xanthid crabs. The toxin is variously used as a defensive biotoxin to ward off predation, or as both defensive and predatory venom. All TTX-bearing organisms show extremely high resistance to TTX, and seem to possess TTX as a biological defense mechanism. Nevertheless, the exact pathway of TTX in the food chain remains unknown, but since the environments of animals which bear this toxin have no known common factor other than the presence of these bacteria, these are believed to be the main source of the toxin (Noguchi 2008; Saoudi et al., 2011).

Several years of research on TTX revealed that toxicity of puffer fishes shows significant individual and regional variations (Sabrah et al., 2006; Noguchi 2008; Arakawa 2010). Most studies reported that the liver of many puffer fish species has a specific TTX uptake mechanism, and TTX introduced into their bodies is first absorbed in the liver and then transferred to the skin through the circulatory system. This inter-tissue transfer and accumulation of TTX are greatly affected by the maturity state of the fish. An alternative explanation is that the toxin is produced by symbiotic or parasitic bacteria which the puffer fish accumulate inside their bodies rather than through feeding. Interestingly, the amount of TTX produced by the bacteria does not account for the accumulation in the fish, which means that bio-concentration occurs in the predator (Katikou et al., 2009; Kalogirou 2010). However, the exact TTX accumulation mechanisms are still unclear and debatable. The reason TTX is produced by the bacteria also remains unknown since it has not been found to be a by-product of any known pathway. Although for the predators of the bacteria the toxin serves as a defense mechanism, it confers no protection to the bacteria themselves. However, evidence also shows that the toxin is an end-product of a biosynthetic pathway rather than a by-product, which means that TTX must have an important function for the bacteria that still needs to be discovered (Simidu 1987; Do 1993; Matsumura 1995; Noguchi 2008; Arakawa 2010; Williams 2010).

De Sousa (2011) stated that TTX has been usually related to the tropics where most of the species known to possess it are found. However, due mainly to the Lessepsian migration, this is no longer the case as TTX possessing species have migrated into other ecosystems. Furthermore, certain studies have shown that there is a correlation between water temperature and the uptake of TTX by organisms. This was tested in 2007 on the puffer fish *Takifugu rubripes* which uptakes TTX into its liver faster with the increase of water temperature. Other studies were carried out subsequently, of which the results agreed that organisms uptake TTX producing bacteria more efficiently and at a faster rate when temperatures increase. For instance, in 2001, *Lagocephalus lunaris*, another species of the Tetrodontidae family, was shown to uptake TTX much faster during warmer months. Even though more investigation is needed to confirm the relation between toxicity and temperature, it can be predicted that as temperatures rise globally and specifically in the Mediterranean, which is very much affected by climate change, the threats which this toxin poses might increase. When this is coupled with easy migration routes and breaking of biological barriers, the need for awareness raising and prevention measures becomes paramount.

2.2.2 Chemistry

Both the chemistry and the structure of TTX (Figure 5) are well known as many researchers have studied its chemical properties, origins, derivatives, microbial source, and mechanisms of actions (Bilecenoglu et al., 2006; Chamandi 2009; De Sousa 2011).

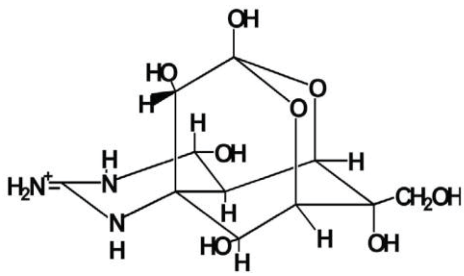

Figure 5. Molecular structure of Tetrodotoxin (Chamandi et al., 2009)

TTX is water soluble, heat resistant, and it can be absorbed through mucous membranes and the small intestine. It inhibits neuromuscular transmission by binding to the voltage-gated sodium channel, where six binding sites have been identified. TTX binds irreversibly to site number one, blocking it and preventing the access of sodium through this channel. Eventually, this toxin leads to the blockage of nerve conduction and therefore to muscle contraction. High concentrations can also lead to cardiac and smooth muscle inhibition. TTX is known to be one of the strongest toxic substances upon ingestion by humans, since it leads to a series of symptoms, including gastrointestinal symptoms such as nausea, diarrhea, vomiting, abdominal discomfort, neurological symptoms such as paresthesia in the face, motor in-coordination, and muscle weakness. Other symptoms include hypotension, hypoxia, slurred speech, and tachycardia. Patients remain lucid during the whole intoxication period (Gosh et al., 2004; Ferreira 2010). No cures for patients who have ingested the toxin exist, but a study conducted by Kendo Matsumura (1995), found that monoclonal antibodies can act against TTX. When limpet hemocyanin was injected into mice prior to being injected by TTX, it acted as an immunogenic and the mice showed no clinical signs of intoxication. This monoclonal antibody was highly specific for TTX and had no cross-reaction to its derivatives such as paralytic shellfish toxin and crude proteins from various organs of puffer fish.

2.2.3 Toxicity of *L. sceleratus*

Most of the studies found around the world have targeted other species of puffer fishes, and therefore publications concerning *L. sceleratus* in particular are relatively scarce. Most of the studies related to its toxicity were conducted in the Mediterranean region due to its negative impact on the fisheries sector. Studies in this region showed that individuals of *L. sceleratus* in the premature stage with sizes of less than 16 cm in length usually do not possess enough TTX to be lethal, but that as an adult, as little as 200 grams of its flesh are lethal to humans (Sabrahet al., 2006; Katikouet al., 2009; Ali et al., 2011). These observations suggest that this puffer fish, just like other species, accumulates TTX as a biological defense agent with time as it matures (Ahasanet al., 2004; Saoudiet al., 2008; Arakawa et al., 2010; Bragdeeswaran & Therasa 2010; Noguchi 2008; Ali et al., 2011). TTX was found mainly in the gonads, liver and intestines of this species, but was also recorded on the skin and occasionally in the muscles (Sabrahet al., 2006; Katikouet al., 2009; Ali et al., 2011; Aydin 2011). The toxin is also present in spawned eggs protecting them from predation. The ovaries (Figure 6) are reported to be strongly toxic (>1000 MU/g tissue), the intestines moderately toxic (100-1000 MU/g tissue) while the liver, muscles and skin are weakly toxic (10-100 MU/g tissue). Moreover, when the fish encounters enemies, its body swells and TTX is excreted from the skin as a repellant.

In a study conducted in Egypt in 2011 about the toxicity of *L. sceleratus*, 57% of the samples examined were toxic, with toxicity varying with size, between organs, and between sexes. TTX was found in the muscles, skin, liver, ovaries, testis, and intestines of the fish, with toxicity reaching its maximum values in the gonads, liver, and intestines. Furthermore, females were found to be generally more toxic than males and the highest toxicity levels were recorded in March, which led the researchers to conclude that toxicity starts to increase as the spawning season approaches. Even though TTX is more concentrated in certain internal organs, the study revealed moderately toxic muscle tissue especially in spring and summer, which further confirms the risk this species poses to public health (Ali et al., 2011).

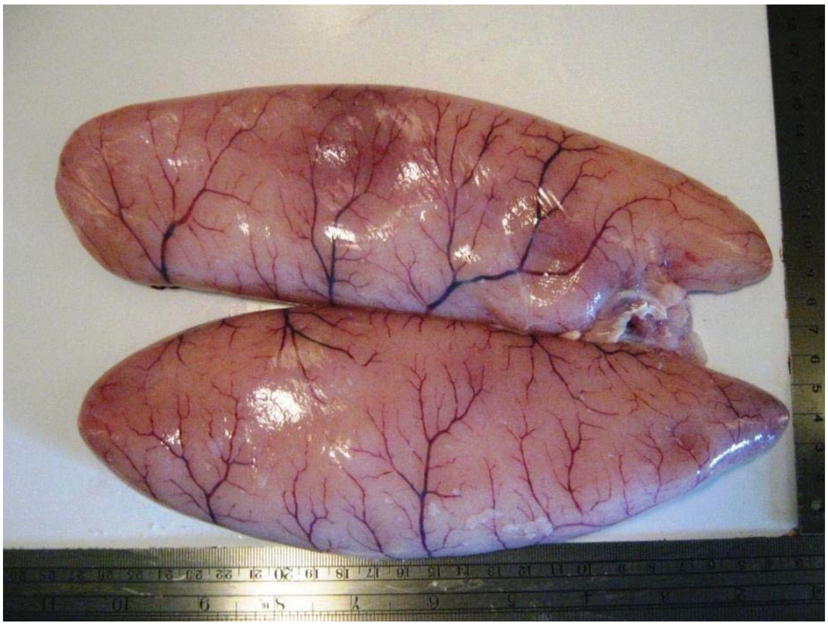

Figure 6. Mature ovaries of *L. sceleratus* (Photo by Marine Resources and Coastal Zone Management Program-Institute of the Environment-University of Balamand, Lebanon, 2011)

A study on the biology and toxicity of *L. sceleratus* (Sabrah 2006) was carried-out in Egypt in the Gulf of Suez between 2002 and 2003 of which the results showed that toxicity increases as the individuals mature and their gonads develop. The most toxic fish were found to be in their spawning stage during the months of April, May, and June. Out of all the fish examined, 24% were found not to be toxic, 32% were weakly toxic (10-100 MU/g tissue), 20% were moderately toxic (100-1000 MU/g tissue), and 24% were strongly toxic (>1000 MU/g tissue). A study on the toxicity of this species held by Katikou et al. near the Rhodes Island in 2009 where the internal organs, muscles and skin were tested for TTX obtained similar results to the two previous studies.

3. World fisheries of *Lagocephalus sceleratus*

Several puffer fish species have been consumed for centuries in certain Asian countries where they are considered a delicacy and where they are prepared by certain trained and licensed chefs who know how to serve the non-toxic parts. For instance, in Japan, there are several different types of puffer fishes, locally known as Fugu, which are labeled as edible (Takeda 1996). Since not all puffer fishes are toxic while others are mildly toxic means that they can be consumed safely. Furthermore, toxicity changes with age, sex, season, and geographical variation (Homaira 2008). The species which contain toxic organs are consumed in certain countries, where they are sliced in such a way as to separate the toxic organs and fast enough as to not allow the toxin to penetrate into the flesh. The chefs who prepare Fugu dishes are required to obtain a license by passing an official exam which can be taken in Japan (Magina 1988). However, although many species of puffer fish are economically important for the fisheries of the countries where they are consumed, *L. sceleratus* in particular, is not among the commercial species due to its high toxicity. Therefore, it is considered as a non-edible species and its consumption poses a great risk to the public.

In the Mediterranean, this invasive species, is being caught as by-catch in relatively significant numbers, has no actual economic value and is therefore directly discarded at sea (EastMed 2010a). Recently, several studies around the Mediterranean have been targeting this species given its negative impact on the fisheries sector. For example, in a study carried out in Cyprus from 2009 to 2010, landings data on *L. sceleratus* were collected from artisanal fishers and it was found that it represented 4% of the weight of the total artisanal catches. It was also found that this species inhabits mainly warmer areas in the South-East coast of Cyprus and that it is not affected by the seabed bottom type. It was concluded that this fish has been able to successfully establish itself due to its rapid growth, reproduction at an early age, adaptation ability, absence of predators and/or competitors, and the fact that it is not a targeted species. Fishers have had to change their methods and gears of fishing and even their fishing grounds in order to reduce the negative impacts of this invasive on their livelihoods (EastMed 2010b).

A thorough search for information related to *L. sceleratus* revealed a lack of publications and reports that address the fishery of the species. This particular fish was never a very important subject for scientific research since in its native areas it possesses no market value and therefore it is of no particular interest. Most of the articles are about puffer fishes as a group or about a few species of economic interest. However, this species was put under the microscope recently by scientists around the Mediterranean where *L. sceleratus* is a new Lessepsian and is considered as one of the worst invasives (Streftaris & Zenetos 2006). Several scientists have launched field assessments to better understand its biological and ecological characteristics. These investigations that require partnerships with the fishing community are facing a number of challenges since in some cases, fishermen are not able to accurately identify this specific species and differentiate it from others. In addition, landing data is hard to obtain as several countries banned its fishing, landing and trade (Kalogirou 2010).

4. Impacts of *Lagocephalus sceleratus* and TTX

As previously mentioned, few studies on the biological characteristics, behavior, and toxicity of *L. sceleratus* were held worldwide. Since its invasion of the Eastern basin of the Mediterranean, it has posed a serious challenge from an ecosystem and fisheries perspective as well as from a health point of view for consumers (IUCN 2000; Galil 2002; IUCN 2008; Oral 2010).

4.1 Impact of *L. sceleratus* on public health

There have been many cases of human intoxication due to the ingestion of several TTX-bearing puffer fish species, mainly in Japan, China, and Taiwan where they have been historically consumed, and where several victims have died (Arakawa 2010; Bragdeeswaran & Therasa, 2010; Monaliza & Samsur 2011). This risk is now also present for consumers in the countries of the Mediterranean where several invasive puffer fish species are now present (Chamandi 2009; Kalogirou 2010). TTX is not destroyed by heat while cooking the fish and intoxication cases are mainly due to lack of awareness among consumers, misidentification of species or due to erroneous traditional conception of detoxification methods. The severity of the intoxication depends on the amount of TTX ingested and death usually occurs after six to twenty-four hours in 60% of the cases, but can even occur after seventeen minutes in certain situations (Aydin 2011). The minimum lethal dose for a 50 kg individual has been estimated to be 10 000 MU (2 mg of TTX), but this may vary depending on the age, health, and sensitivity of the person, and therefore does not apply in all cases. In addition, and since the concentrations of TTX differ among different fish, it is very hard to determine one standard dose. Immediate gastric lavage is needed after four hours of ingestion, followed by symptomatic and supportive treatments, activated charcoal, saline cathartic, and clinical care for some time (Haque 2008; Ferreira 2010). However, there is no fundamental treatment which eliminates the toxin completely from the body (Arakawa 2010) and no antidotes or antitoxins exist for TTX. If a patient is seriously intoxicated, the only treatment which is available is artificial respiration until the body detoxifies itself.

In several Asian countries where puffer fishes are consumed, TTX poisoning is the most commonly reported fish poisoning (Chua 2009). In Japan, even though it has been known for years that the liver, which is commonly known as ‘kimo’, is very toxic, some individuals dare to consume it. The intoxication cases have been decreasing with time, with puffer fish poisoning constituting 12.5% of all food poisonings in 1890, 16% in 1895, 20% in 1900, 12% in 1905, and 4% in the1950s. By the year 2000, this number had decreased to 2%. The first data published by the Japanese Ministry of Health was in 1952 when it was recorded that 73% of poisonings happened at home and 15% in restaurants, with the rest occurring in hotels and even hospitals. Even today, more than three-fourth of the cases occur at home (West 2009). These results of frequent cases of poisoning has led the Japanese Ministry of Health and Welfare (http://www.mhlw.go.jp/english/) to publish a list of edible puffer fishes in 1983 excluding *L. sceleratus*. This list was updated in 1993 and 1995, where the liver of all puffer fish was banned from being sold in the market or served in restaurants. Regardless of the fact that only trained and licensed cooks have the right to prepare the fish in Japan, still cases of poisoning occur due mostly to its preparation by uncertified handlers (Ahasan 2004; Chamandi 2009). A 2002 publicized case in Bangladesh reported that thirty-seven patients of eight different families were all suffering from TTX induced symptoms. They had all bought puffer fish from a market in their village and none of them was aware of the risks. Eight out of the thirty-seven patients died within a few hours and the cause of death was declared as respiratory muscle paralysis which led to respiratory failure (Ahasan 2004). Regarding *L. sceleratus*, it is listed among the 22 toxic puffer fishes in Japan, where it is commonly known as Okinawafugu, and where its consumption is completely banned (Arakawa 2010). Also in Malaysia, where *L. spadiceus* and *L. lunaris* are consumed, the consumption of *L. sceleratus* is illegal due to several cases of severe intoxication (Monaliza & Samsur2011).

In the Mediterranean, several cases of poisoning have been recorded as *L. sceleratus* is marketed regardless of the risk it poses to public health. Its large size might be one of the reasons behind this species being sold. There have already been 13 recorded cases of death in the Eastern Mediterranean as well as other cases of intoxication (Chamandi et al., 2009; Kalogirou 2010). From the time *L. sceleratus* settled in the Mediterranean, it is being sold and consumed in Egypt, where it is now considered a delicacy irrespective of its ban by Egyptian law. In Turkey, where landings have also been banned, fishers still sell it illegally and Turkish fishers readily consume it (Aydin 2011). Moreover, this species is also consumed in Lebanon by some fishers and a small number of consumers ignorant of the health threats it poses where several cases of unofficial intoxication have been reported in that country after eating *L. sceleratus*. The only official record was in 2008 when a 68 year old woman complaining of limb weakness and dyspnea was brought to a hospital in Beirut. The family revealed after questioning that she had eaten a half-cooked liver of *L. sceleratus* (Chamandi et al., 2009). Even though in that particular case the woman survived, the local media records seven cases of death in the past few years in Lebanon due to consumption of puffer fishes. As a result, the Lebanese authorities banned in 2011 the fishing, selling and consuming of all puffer fishes including *L. sceleratus*. Fish consumers in the Eastern Mediterranean are becoming increasingly concerned about the availability of those fishes in the market since it is hard to separate small-sized individuals from other commercial species of the same size resulting in accidental consumption.

4.2 Impact of *L. sceleratus* on fisheries

No real, scientific assessment to evaluate the direct impact of *L. sceleratus* on the fisheries sector in the Indo-Pacific region where this species is native was found. Its damage to fishing gears was mentioned in several un-official reports, but it is presumed that its impact in those areas is minimal since it is part of the ecosystem.

According to Kalogirou 2010, it is agreed that the socio-economic impact of this alien species on the local fisheries of the Eastern Mediterranean countries is significant. Complaints from local fishers have become frequent in Egypt, Lebanon, Cyprus, Turkey and Greece amongst others where the destruction of nets due to entangling or to predation by *L. sceleratus* on already entangled fish is common (Kalogirou 2010). *L. sceleratus* is considered a major nuisance by fishers since it damages fishing gear by attacking fish caught in nets and lines, along with reducing local stocks of squids and octopus through predation. This species can easily cut lines and nets using its strong teeth (Figure 4). All of the above is affecting the well-being of the fishing community by increasing the time spent fishing, the mending and replacing of damaged gears and cleaning nets from puffer fishes and their remains. One example of this species' impact comes from Fethiye Bay in Turkey, where after five minutes of recreational fishing, three fishing lines were broken, ten hooks went missing, and a *L. sceleratus* weighing 1 kg was captured. In one study, 52 long-line hooks were found in 33 stomachs of these fish and with the time spent to clean the gears from discarded fish, the negative impact was even higher. Furthermore, reductions of commercial catches of *Sepia officinalis*, which can be related to the presence of *L. sceleratus* have been observed (Kalogirou 2010).

Fishers in Cyprus have been facing the same problems and have already been forced to modify their fishing techniques and habits (EastMed2010b). Similarly, Lebanese fishers are complaining from the damage caused to their gears and their catch. Some have already started adding a metallic wire at the end of their fishing lines to prevent the fish from biting through them therefore reducing the loss of hooks and weights. On the other hand, fishing net material has not yet been modified and it is not clear if other net manufacturing techniques will reduce tearing. When it comes to pot users, complaints reveal that this species either eats the catch or keeps fish away from entering the pots. For all of these reasons, and the fact that it is not marketable, *L. sceleratus* has established itself as a major nuisance due to the costs incurred to replace lost gear, repair existing ones, and to the additional effort required by the fishers to land a catch of commercial value. These facts call for immediate action. Already, Turkish, Greek, Cypriot, Lebanese and Egyptian Ministries of Agriculture as well as others, have banned the fishing and selling of this species. However, additional measures are needed to develop plans and approaches which decrease *L. sceleratus* impacts on fisheries. Preventive measures include fishing in deeper grounds (>60 m), attaching the hooks to steel lines to prevent their loss to biting, as well as producing nets with stronger and more resistant material (Kalogirou 2010).

5. Legislation related to *Lagocephalus sceleratus* throughout the world

Intoxication due to consumption of puffer fish species is well recognized and the reasons are known worldwide. Therefore several governments have already banned or restricted the consumption of any products related to puffer fishes. In addition awareness campaigns are being launched in several countries to expose the threat of this species to consumers but illegal consumption still occurs and therefore intoxication risk still poses a serious threat. Nevertheless, several countries still show lack of regulation and awareness increasing the risk of exposing their consumers to TTX.

In Japan, the government collects data about Fugu poisoning from physicians, who are required to report every case. It was in 1947 that the Food Sanitation Law #37 was passed, which provided that "No person shall sell, handle, manufacture, import, process, use, prepare, store, or display with intent to sell any food . . . [that] contain[s] or bear[s] toxic or injurious substances. . . provided, however, that this provision does not apply to the cases which are prescribed by the Minister of Health, Labor, and Welfare as not injurious to human health." In 1958, another law was passed which require cooks to obtain a license in order to be allowed to serve Fugu. Today, the Japanese Criminal Code can provide a fine or up to five years of imprisonment for serving puffer fish without a license and causing a death or injury (Noguchi & Arakawa 2008; West 2009; Arakawa et al., 2010). In addition, puffer fish imported to Japan requires a sanitary or health certificate which food sanitation inspectors must examine to make sure it complies with the Food Sanitation Law. Fugu may be imported either unprocessed or gutted (as long as the identification of each fish is not impaired). Fish must be tagged with an official certificate from the exporting country identifying it by scientific name, fishing ground, and attesting to the fact that it received proper sanitary handling. If the fish is frozen, it must have been deep frozen and stored at a temperature below -10 °C. In addition, each fish should be individually frozen for easy identification. If this is not possible, fish may be frozen in blocks, but each fish's back and belly must be visible for species identification. If official certificates are incomplete or detached, or if the cargo is mixed with other species restricted from import, the cargo may be returned to the exporter. Imports of the same or other Fugu species harvested in other waters must be negotiated with the Japanese Government to determine certificates of toxicity, fishing ground, species, etc. (Ababoush 2005; Noguchi & Arakawa 2008).

In Thailand, puffer fish imports, exports, and production were completely banned in 2002 after six cases of death in that year due to puffer fish poisoning. According to the Health Minister Sudarat Keyuraphan in 2003, "differentiating between properly cleaned puffer fish and toxic puffer fish is too difficult; therefore a complete ban is the only solution to the problem". The Food and Drug Administration created teams of people which inspect fish markets and restaurants in Thailand. Those who fail to comply can be either fined or imprisoned for up to two years. However, illegal selling of this fish still occurs (The Age 2003).

In 1989, the Food and Drug Administration (FDA) in the United States, after four years of negotiations, reached an agreement with the Japanese Ministry of Health and Welfare to allow the import of puffer fish from Japan under certain conditions. The agreement permitted only the import of the meat, skin, and testicles of tiger puffer fish (*Takifugu rubripes* or torafugu), which must be prepared in a specific facility and certified. In addition, this product can be sold only to restaurants which belong to the Torafugu Buyers Association. In case puffer fish are imported into the US by a country other than Japan or by Japanese which are not complying with the terms of the 1989 agreement, the individuals involved become subject to the FDA Import Alert #16–20, which allows for their detention. In Canada, imports of *L. sceleratus* and puffers of the family Tetrodontidaeare are prohibited by the law for “Regulatory Requirements for Fish Import License Holders”, section 4.2.1 (www.inspection.gc.ca).

According to Corrigendum Regulation (EC) No 854/2004 of the European Parliament of the Council of 29 April 2004 poisonous fish belonging to the Tetrodontidae family are not placed on the market in Europe. This regulation laid down specific rules for the organization of official controls on products of animal origin intended for human consumption (http://eur-lex.europa.eu). In Lebanon, the

Ministry of Agriculture issued a decree in July 2011 (Ministry of Agriculture, Lebanon, Decree 676/1) which bans fishing, selling and consuming *L. sceleratus*. In Turkey, the Ministry of Food, Agriculture and Livestock issued a ban on the fishery of *L.sceleratus* (Bilecenoğlu 2012) and the Egyptian, Cypriot, and Greek governments have all banned the fishing and selling of puffer fishes (Kalogirou 2010).

6. Commercial applications of *Lagocephalus sceleratus*

L. sceleratus is considered a highly toxic species and therefore it is not targeted for human consumption even in the countries where other species of puffer fishes are being consumed. Consequently, it is not landed on the world markets as a commercial species and does not have any economic value.

6.1 *L. sceleratus* as a food item

Puffer fish species have been shown to become non-toxic when their diets are TTX-free and when they are bred in an environment where the organisms which bear TTX are not present in the food web. These farmed fish are fed with food composed of sardines and mackerel considered free of the *Vibrio* bacteria that is usually found in their natural diets (Arakawa 2010). Of the tens of thousands of tons of Fugu that are consumed each year in Japan, many come from farms, where the fish are not allowed to incorporate the toxin through their diets. The farmed puffer fishes are fed fresh fish until they reach market size. Aquaculture of several species of Fugu is practiced in Japan as full-scale farming by artificial insemination but no references to the farming of *L. sceleratus* in particular were found. It can be assumed that due to its high toxicity and absence of any economic value, this species is not among those which are cultured (Yang 2007; Takeda 1996). In the Mediterranean, farming of puffer fish is not practiced.

As previously stated, the different internal organs of *L. sceleratus* and even its muscles and skin may contain lethal doses of TTX. Nevertheless, several studies have revealed that younger specimens (Total Length < 16cm) contain low amount of TTX or even no toxin at all (Sabrahet al., 2006; Saoudiet al., 2008; Katikouet al., 2009; Simon et al., 2009; Ali et al., 2011). *L. sceleratus* individuals range from not toxic at all to strongly toxic and this difference is dictated by size, with smaller individuals being less toxic than large ones and with toxicity increasing with age (Section 2). As much as 24% of the individuals studied by Sabrah (2006) belonging to maturity stage I & II (<16cm) were too young to contain any toxin. Those results were corroborated by the study held by Katikou (2009) that also showed that individuals of maturity stage I & II were not toxic. Further investigations are therefore needed in the Mediterranean in order to determine the exact size range at which toxicity becomes lethal in the various organs. If, and only if, a certain range can be identified as non-toxic, then several initiatives for commercial use can be foreseen. In this way, a capture based aquaculture industry and/or a fishery can be established where juveniles are harvested and commercialized.

6.2 *L. sceleratus* as a non-food item

Based on the Japanese regulations, which are considered as the most elaborate regarding puffer fish species consumption, *L. sceleratus* is labeled as a non-edible fish due to its high toxicity. Nevertheless, this species can have certain commercial applications mostly due to its characteristic of accumulating high TTX concentrations. Several initiatives isolated TTX from puffer fish species and evaluated the potential commercial applications of the toxin. It is assumed that the same will apply to *L. sceleratus*.

6.2.1 Potential medical uses of TTX

TTX, which is a particular sodium channel blocker, can play a very significant role in neurophysiology and in other fields as a research reagent (Bhakuni & Rawat 2005; Noguchi 2008). Several researchers have conducted studies to evaluate the analgesic activity of TTX for cancer patients. The results showed that TTX can be used as an effective pain reliever. It relieved severe, treatment resistant cancer pain in the majority of the patients involved in the studies. In addition, blocking of fast Na+ channels has potential medical uses in treating some cardiac arrhythmias. Furthermore, TTX has proven useful in the treatment of pain from such diverse problems as migraines, neuralgia, rheumatism, and heroin withdrawal (Matsumura 1995; Schwartz et al., 1998; Dewick 2002; Sowerbutt 2004; Stummann 2005; Hagen 2007; Matsumoto & Tanuma 2009). Given the proven benefits of TTX in the pharmaceutical industry, the establishment of laboratories in East Mediterranean countries by interested companies for the purification and subsequent use of the toxin in the pharmaceutical industry can be considered. Eastern Mediterranean countries could then establish a fishery for *L. sceleratus* specifically oriented towards the pharmaceutical sector. Such an option would create many employment opportunities in the region, but more importantly, it will create a fishery that will yield economic benefits to the fishers and control wild *L. sceleratus* populations through increased fishing pressure. Countries nevertheless would have to introduce very strict legislation to ensure that the fish caught is not landed on the market nor consumed in any way.

6.2.2 Potential Aquarium Use

Puffer fishes are commonly used in aquariums all around the world, regardless of their toxicity. Since they are increasing in number in the Eastern Mediterranean region and are spreading rapidly, it would be relatively easy for fishers to catch them in large enough quantities for the aquarium trade. The fish could be caught at any size according to market demand and would become available for household tanks. Nevertheless, this is not considered to have a significant impact on the wild populations of *L. sceleratus.* As in the pharmaceutical industry trade, strict regulations and monitoring are necessary to ensure that the public health is not endangered.

7. Recommendations

Given the lethal toxicity of *L. sceleratus,* and the strict regulations passed around the world preventing its fishing and consumption, coupled with the little knowledge available about its biology and its bio-concentration of TTX, it is not recommended at this stage to consider marketing the fish to consumers. Even though juveniles do not appear toxic, allowing their consumption will pose unnecessary risk to consumers given the difficulty in monitoring landings an enforcing size limits. Nevertheless and as extensively described in the report, *L. sceleratus* poses threats on several levels in the Eastern Mediterranean countries and therefore potential solutions to the problem should be explored. Even-though the aquarium and capture-based aquaculture industries may provide partial solutions, the best option lies in creating, and in association with pharmaceutical companies, multidisciplinary laboratories in Mediterranean countries with the main objective of isolating TTX from puffer fishes including *L. sceleratus* and investigating the toxin's potential use in the pharmaceutical industry. Such an option would create many employment opportunities in the region, starting with the fishers, passing through the chemist/toxicologist to the medical researchers and ending with the use of the toxin in everyday medical treatment. More importantly, these laboratories will create a fishery that will yield economic benefits to the fishers and control wild populations through increased fishing pressure. However, such an initiative requires very strict permitting and regulatory processes in the countries where such a fishery might be established.

Currently though, much more information is required about the biology and ecology of *L. sceleratus* and its bio-concentration of TTX before action is taken. Based on the reviewed literature, the following is recommended for the Eastern Mediterranean:

- Initiate investigative studies on the biology and population dynamics of *L. sceleratus.*
- Evaluate the impact of *L. sceleratus* on ecosystem health and equilibrium.
- Establish the concentrations of TTX in the different organs and tissues of, and according to the size of *L. sceleratus*.

- Investigate the mechanisms regulating the bio-concentration of TTX in the organs and tissues *of L. sceleratus.*
- Assess the economic value and potential of TTX as a pharmaceutical agent on the world market.
- Develop resilient fishing lines and nets to withstand the biting power of *L. sceleratus* to minimize economic losses to the fisherman.
- Identify the best techniques, fishing areas and period of the year where the fishers can maximize his/her catch of commercially valuable species and reduce the by-catch of *L. sceleratus.*
- Create a forum where scientists, fishery managers, fishers and the community at large can exchange knowledge and share experiences related to *L. sceleratus.*
- Launch awareness raising campaigns targeting coastal Mediterranean communities on the identification of *L. sceleratus* and its risk to public health.

8. Conclusion

L. sceleratus is expected to have significant economic, ecological, and social impacts on Eastern Mediterranean countries. This calls for extensive attention and intervention, be that on the scientific, adaptation and/or mitigation levels. The scarcity of research conducted to date on *L. sceleratus* requires tackling the issue from a socio-economic as well as an ecological perspective. Solutions for reducing the negative impacts of this Lessepsian migrant are available, but a better understanding of the biology and ecology of the fish including the potential commercial use of TTX is required. Needless to say, Lessepsian migration is not only expected to continue but to increase in intensity as a result of climate change. The impacts of such migrations are expected to increase. Identification and monitoring of invasives at an early stage will better prepare fisheries managers to come-up with preventive/adaptive measures and possibly economic uses for the new migrants. Furthermore, an action plan developed and endorsed by Mediterranean countries should be formulated to minimize, if not eradicate, the entry of invasive species arriving from the Red Sea within the Canal itself and before they establish viable populations in the Mediterranean.

9. References

Ababouch, L., Gandini, G., Ryder, J. (2005). Causes of Detention and Rejections in International Fish Trade. FAO Fisheries Technical Paper. 473.

Ahasan, H., Mamun, A., & Karim, S. (2004). Paralytic Complications of Puffer Fish (Tetrodotoxin) Poisoning. *Singapore Medical Journal, 45* (2), 73-74.

Akyol, O., Unal, V., Ceyhan, T., Bilecenoglu, M. (2005). First Confirmed Record of *Lagocephalus sceleratus* (Gmelin 1789) in the Mediterranean Sea. *Journal of Fish Biology*. 66 (4): 1183-1186.

Ali, A.E., Gomaa, M., Othman, H. (2011). Toxicological Studies on Puffer Fishes, *Lagocephalus sceleratus* and *Amblyrhynchotes hypeslogenion* in Suez Gulf, Red Sea, Egypt. *Egyptian Journal of Aquatic Research*. 37(2):123-130.

Arakawa, O., Hwang, D.-F., Taniyama, S., & Takatani, T. (2010). Toxins of Pufferfish That Cause Human Intoxications. *Coastal Environmental and Ecosystem Issues of the East China Sea* , 227-244.

Arguelles, L., Zilletti, B. (2008). A Perspective of Climate Change and Invasive Alien Species. Convention on the Conservation of European Wildlife and Natural Habitats, 2nd Meeting of the Group of Experts on Biodiversity and Climate Change. Strasbourg, 1-31.

Aydin, M. (2011). Growth, Reproduction and Diet of puffer fish (*Lagocephalus sceleratus* Gmelin, 1789) from Turkey's Mediterranean Sea Coast. *Turkish Journal of Fisheries and Aquatic Sciences*. 11:589-596.

Bernardi, G., Golani, D., & Azzurro, E. (2010). The genetics of Lessepsian bioinvasions. *Fish Invasions of the Mediterranean Sea* , 71-84.

Bhakuni, D.S.& Rawat, D.S. (2005). Bioactive Marine Natural Products. *Anamaya Publishers*. 178-182.

Bilecenoglu, M., Kaya, M., Akalin, S. (2006). Range Expansion of Silverstripe blaasop, *Lagocephalus sceleratus* (Gemlin 1789), to the Northern Agean Sea. *Aquatic Invasions*. 1(4): 289-291.

Bragadeeswaran, S., & Therasa, D. (2010). Biomedical and Pharmacological Potential of Tetrodotoxin-producing bacteria isolated from the Marine Puffer Fish. *The Journal of Venomous Animals and Toxins including Tropical Diseases, 16* (3), 421-431.

Burgiel, S. & Muir, A. (2010) Invasive Species, Climate Change and Ecosystem-Based Adaptation: Adressing Multiple Drivers of Global Change. Global Invasive Species Program. 1-56.

Carpentieri, P., Lelli, S., Colloca, F. C., Mohanna, C., Bartolino, V., Moubayed, S., Ardizzone G.D. (2009). Incidence of lessepsian migrants on landings of the artisanal fishery of South Lebanon. *Journal of Marine Biodiversity Records*. 2:1-5.

Chamandi, S. C., Kallab, K., Mattar, H., & Nader, E. (2009). Human Poisoning after Ingestion of Puffer Fish Caught from Mediterranean Sea. Case Report - USEK Medical School - Lebanon.

Chua, H., & Chew, L. (2009). Puffer Fish Poisoning: A Family Affair. *Medical Journal Malaysia, 64* (2).

Corsini-Foka, M., Margies, P., Kondilatos, G., & Economidis, P. S. (2005). Lessepsian migration of fishes to the Aegean Sea: First record of *Tylerius spinosissimus* (Tetraodontidae) from Rhodes. *Cybium* , 347-354.

Corsini-Foka, M., Margies, P., Kondilatos, G., & Economidis, P. (2006). *Torquigener flavimaculosus* Hardy and Randall, 1983 (Pisces: Tetraodontidae) off Rhodes island marine area: a new alien fish in the Hellenic waters. *Mediterranean Marine Science, 7* (2), 73-76.

De Sousa, M.L. (2011). Occurrence of Tetrodotoxin Producing Bacteria on Marine Gastropods of the Northern Coast of Portugal. University if Porto, Portugal. p. 80.

Dewick, P. M. (2002). Medicinal Natural Products, A biosynthetic Approach. *John Wiley and Sons,* Second Edition. 396-403.

Do, H.K., Hamasaki, K., Ohwada, K., Simidu, U., Noguchi, T., Shida, Y., Kosure, K. (1993). Presence of Tetrodotoxin and TTX-Producing Bacteria in Freshwater Sediments. *Applied and Environmental Microbiology Journal.* Vol. 59, No. 11, p 3934-3937.

EastMed, 2010a. Report of the Sub-Regional Workshop on Collection and Organization of Data. Scientific and Institutional Cooperation to Support Responsible Fisheries in the EasternMediterranean. GCP/INT/041/EC – GRE – ITA/TD-03

EastMed, 2010b. Report of the Sub-Regional Technical meeting on the Lessepsian migrationand its impact on Eastern Mediterranean fishery. GCP/INT/041/EC – GRE – ITA/TD-04.

FAO Fisheries and Aquaculture Department (2010). The State of World Fisheries and Aquaculture. p. 218.

Ferreira, T.R., Correa, I.R., Hoshino, S., Silva, C., Pardal, P. (2011). Envenomation Caused by the Consumption of Puffer Fish Liver. *Toxicological Information Center*. 1-5.

Galil, B. S., & Zenetos, A. (2002). A Sea Change - Exotics in the Eastern Mediterranean. Invasive Aquatic Species in Europe. Distribution, Impact and Management, 325-336.

Golani, D. (1998). Impact of Red Sea fish migrants through the Suez Canal on the aquatic environment of the Eastern Mediterranean. Bulletin of Yale School Forest. Environmental Studies, 103: 375-387.

Golani, D., & Azzurro, E. (2007). Genetic Bottlenecks and successful Biological Invasions: The Case of recent Lessepsian Migrant. *Biology Letters, 3*, 541-545.

Gosh, S., Hazra, A. K., Banerjee, S., & Mukherjee, B. (2004). The seasonal toxicological profile of four puffer fish species collected along Bengal coast, India. *Indian Journal of Marine Sciences, 33* (3), 276-280.

Hagen, N. A., & Fisher, K. M. (2007). An Open- Label, Multi- Dose Efficacy and Safty Study of Intramuscular Tetrodotoxin in Patients with Severe Cancer- Related Pain. *Journal of Pain and Symptoms Management, 34* (2), 171-182.

Haque, M. A., Islam, Q. T., & Ekram, S. (2008). Puffer Fish Poisoning. *The Journal of Teachers Association*, *21* (2), 199-202.

Homaira,N., Rahman, M., Luby, S., Rahman, M., Haider, M.S., Faruque, L.I, Khan, D., Parveen, S., Gurley, E. (2010). Multiple Outbreaks of Puffer Fish Intoxication in Bangladesh 2008. *Centers for Disease Control and Prevention (CDC)*. 1-9.

IUCN - ISSG, (2000). Marine Menace, Alien Invasive Species in the Marine Environment. 1-32

IUCN - ISSG, (2008). Aliens. 27:1-30.

Kalogirou, *S*., Corsini-Foka, M., Sioulas, A., Wennhage, H., Pihl, L. *(*2010*)*. Diversity, structure and function of fish assemblages associated with *Posidonia oceanica* beds in an area of the Eastern Mediterranean Sea and the role of non-indigenous species. Journal of Fish Biology, 77:2338-2351.

Kasapidis, P., Peristeraki, P., Tserpes, G., & Magoulas, A. (2007). First record of the Lessepsian migrant Lagocephalus sceleratus (Gmelin 1789) (Osteichthyes: Tetraodontidae) in the Cretan Sea (Aegean, Greece). *Aquatic Invasions, 2* (1), 71-73.

Katikou, P., Georgantelis, D., Sinouris, N., Ptesi, A., Fotaras, T. (2009). First Report on Toxicity Assessment of the Lessepsian Migrant Puffer Fish *Lagocephalus sceleratus* (Gmelin, 1789) From European Waters (Agean Sea, Greece). *Toxicon*, 54: 50-55.

Maiyza, I., Said, M., & Kamel, M. (2010). Sea Surface Temperature Anomalies in the South Eastern Mediterranean Sea. *JKAU: Marine Science, 21* (1), 151-159.

Matsumoto, T., & Tanuma, D. (2009). Plasma Protein Binding of Tetrodotoxin in the Marine Puffer Fish. *Toxicon.* 55(2-3): 415-420.

Matsumura, K. (1995, a). Reexamination of Tetrodotoxin Producion Bacteria. *Applied and Environmental Microbiology Journal*. Vol 61, No. 9. p. 3468-3470.

Matsumura, K. (1995, b). A Monoclonal Antibody Against Tetrodotoxin that Reacts to the active Group for Toxicity. *European Journal of Pharmacology, Environmental Toxicology and Pharmacology, 293* (1), 41-45.

Monaliza, M.D. & Samsur M. (2011). Toxicity and Toxin Properties Study of puffer fish collected from Sabah Waters. *Health and Environment Journal*. 2(1):14-17.

Noguchi, T., & Arakawa, O. (2008). Tetrodoxin - Distribution and Accumulation in Aquatic Organisms and Cases of Human Intoxication. *Marine Drugs, 6*, 220-242.

Oral, M. (2010). Alien Fish Species in the Mediterranean - Black Sea Basin. *Journal of Black Sea/ Mediterranean Environment*. 16(1):87-132.

Ozturk, B. (2010). Draft Document on the Alien Species in the Mediterranean and the Black Sea. General Fisheries Comission for the Mediterranean, *Scientific Advisory Committee*. GFCM-SAC 12.

Pancucci-Papadopoulou, M.A., Corsini-Foka, M., Raitsos, D.E. (2011). Alien Invasions in Climatic Changes: The South-Easteran Agean Experience. Proceedings of the 12^{th} International Conferences on Environmental Science and Technology. Rhodes, Greece.

Peristeraki, P., Lazarakis, G., Skarvelis, C., Georgiadis, M., Tserpes, G. (2006). Additional Records on the Occurance of Alien Fish Species in the Eastern Mediterranean Sea. *Mediterranean Marine Science*. 7/2:61-66.

Sabrah, M., El-Ganainy, A., & Zaky, M. (2006). Biology and toxicity of the Puffer fish *Lagocephalus Sceleratus* From the Gulf of Suez. *Egyptian Journal of Aquatic Research*, 32 (1), 283-297.

Saoudi, M., Abdelmouleh, A., Kammoun, W., Ellouze, F., Jamoussi, K., El Feki, A. (2008). Toxicity Assessment of the Pufferfish *Lagocephalus lagocephalus* from the Tunisian coast. *Comptes Rendus Biologies*, 331: 611-616.

Saoudi, M., Messarah, M., Boumendjel, A., Abdelmouleh, Kammoun, W., Jamoussi, K., El Feki, A. (2011). Extracted Tertodotoxin from Puffer fish Lagocephalus lagocephalus induced hepatotoxicity and nephrotoxicity to Wistar rats. *African Journal of Biotechnology*, 10(41): 8140-8145.

Schwartz, D. M., Field, H., Duncan, K., Jones, M. (1998). Experimental Study of Tetrodotoxin, Long Acting Topical Anesthetic. *American Journal of Ophthalmology, 125* (4), 481-487.

Simidu, U., Noguchi, T., Hwang, d., Shida, Y., Hashimoto, K. (1987). Marine Bacteria Which Produce TTX. *Applied Environmental Microbiology Journal*. Vol. 53, No. 7. p. 1714-1715.

Simon, K., Mazlan, A., & Usup, G. (2009). Toxicity of Puffer Fishes (Lagocephalus wheeleri Abe, Tabeta and Kitahama, 1984 and Lagocephalus sceleratus Gmelin, 1789) from the East Coast Waters of Peninsular Malaysia. *Journal of Biological Sciences, 9* (5), 482-487.

Sowerbutt, C. (2004). Puffer Fish Toxin Could Change the Treatment of Cancer Pain. *Powerful Body Peaceful Mind* .

Streftaris, N. & Zenetos A. (2006). Alien Marine Species in the Mediterranean - The 100 'Worst Invasives' and their Impact. *Mediterranean Marine Science*, 7(1):87-118.

Stummann, T. C., & Salvati, P. (2005). Tetrodotoxin for Moderate to Severe Cancer Pain: A randomized, Double Blind, Parallel Mutlicenter Study. *European Journal of Pharmacology, 510* (3), 197-208.

Takeda, A. (1996). "Blowfish and Trade (BLOWFISH Case).D.C.: American University, The School ofInternational Service, 1.

Torcu Koc, H., Erdogan, Z., & Ustun, F. (2010). Occurence of the Lessepsian migrant, *Lagocephalus sceleratus* (Gmelin 1789) (Osteichtyes: Tetraodontidae), in Iskenderun Bay (north-Eastern Mediterranean, Turkey). *Journal of Applied Ichthyology, 27*, 148–149.

West, M. (2009). Haley and the Blowfish. *Washington University Global Studies Law Review*. 8:427-442.

Williams, B. (2010). Behavioral and chemical ecology of marine organisms with respect to tetrodotoxin. *Marine Drugs*, 8, 381-398.

Yaglioglu, D., Turan, C., Erguden, D., Mevlut, G. (2011). Range Expansion of Silverstripe Blaasop, *L. sceleratus* (Gmelin, 1789) to the NorthEastern Mediterranean Sea. *Biharean Biologist*. Vol. 5(2), p 159-161.

Yang, G., Bao, B., & Peatman, E. (2007). Analysis of the composition of the bacterial community in puffer fish. *Aquaculture, 262* (2-4), 183-191.

Zenetos, A., Cinar, M., Papadopoulou, M. P., Harmelin, J., Furnari, G., Andaloro, F., et al., (2005). Annotated list of marine alien species in the Mediterranean with records of. *Mediterranean Marine Science, 6* (2), 63-118.

Beneficiary countries

Countries with waters included in the GFCM Geographical Sub-Areas (GSAs) 19-20 and 22-28

Donors

Greece

- Ministry of Foreign Affairs
- Ministry of Rural Development and Food

Italy

- Ministry of Agriculture Food and Forestry Policies

European Community

- Directorate General of Maritime Affairs and Fisheries (DG-MARE)

Hellenic Ministry of Foreign Affairs

Hellenic Ministry of Rural Development and Food

ITALIAN MINISTRY OF AGRICULTURE, FOOD AND FORESTRY POLICIES

FAO – EastMed Project

1 Androu str. 112 57 Athens Greece
Tel: +30 210 8847960 Fax: +30 210 8837600
E-mail: Eastmed@fao.org http: //www.faoeastmed.org