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MARIE SKŁODOWSKA-CURIE ACTIONS

Individual Fellowships (IF)
Call: H2020-MSCA-IF-2017

PART B

"PARASITES OF MPA"

This proposal is to be evaluated as:

[Standard EF]

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List of Participants

Participants	Legal Entity Short Name	Academic (tick)	Non-academic (tick)	Country	Dept./ Division / Laboratory	Supervisor	Role of Partner Organisation
Beneficiary							
- University Pierre and Marie Curie	UPMC	yes	-	France	Observatoire Océanologique de Banyuls-sur-Mer (OOB)	Yves Desdevises	
Partner Organisations							
- University of Montpellier	UM	yes	-	France	CIRAD AGIRS, Doctoral School GAIA	Serge Morand	Hosting secondments
- Natural Marine Reserve of Cerbere-Banyuls	NMRCB	-	yes	France	-	Frederic Cadene	Intersectoral mobility

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1. Excellence

1.1 Quality, innovative aspects and credibility of the research

Introduction and State-of-the-art

Marine protected areas (MPA) can deliver considerable and rapid benefits for both conservation and fisheries management[1]. Although hundreds of scientific studies have explored the details of the ecosystem's successes in MPAs[1], virtually no attention has been given to the influence of parasites[2]. This may be substantial, as a primary effect of marine reserves is to increase the density of an exploited population within the reserve relative to open-access areas, which may facilitate parasite transmission[2]. Empirical studies have suggested that MPAs increase parasite populations[3] and many conservation practices are associated with parasite-related problems[4]. There is a good example of a mortality event in sea scallops caused by sponge, polychaete, and prokaryote infection that affected an MPA, but was not observed in nearby fished areas. However, other outcomes are possible. Overfishing may increase stress level in fish populations and parasites can synergistically exacerbate fish mortality. McCallum et al.[2] argued that in some cases the combination of a pathogen and a marine reserve may even produce higher harvests than would be obtained by the reserve in the absence of the pathogen.

Although knowledge accumulated on parasites of MPAs has established some tendencies in host-parasite relationships, the role of parasites in the early life-history stages of marine fish in protected areas is far from clear. Studies of marine fish on larval and juvenile stages showed that those are susceptible to a wide range of predators, and predation is among the dominant factor influencing fish survival during the early life-history stages[5,6]. However, predation is likely the ultimate result of a process precipitated by different agents that make some individuals more susceptible to predation by lowering body condition, growth and performance[5,6]. These agents potentially include starvation, disease and parasitism[5,6]. Currently, little is known on the importance of these agents on the condition of marine fish on the early life-history stages[6] and even no research has been carried out on parasites of the fish early life-history stages in MPAs. At the same time, understanding the processes that influence individuals to survive reproduction was established as a fundamental goal in population ecology[7]. *Therefore, the present project focuses on the host-parasite relationships in fish early life-history stages to estimate the effect of MPAs on fish stock recovery in protected and adjacent open-access waters.*

From a fisheries benefit, a measurable effect of a MPA is expected to be the export of biomass to neighbouring fished areas through adult and/or larval spillover. However, the potential of reserves to provide fisheries and conservation benefits to adjacent waters remains heavily discussed[8]. Although the use of molecular data has become a common method in the past 20 years to track fish movements, no single technique or suite of techniques has been established to study fish stock structure[9]. Data on parasite communities (species richness, diversity, abundance and genetics) represent a powerful tool to assess fish host population dynamics because parasites are more sub-structured than their hosts[10]. *We attempt to develop multidisciplinary approach integrating data on parasite community and parasite population genetic structure to assess spillover effect.*

The project will focus on the Mediterranean grey mullets (Mugilidae) in the Banyuls-Cerbère MPA, the oldest French protected area, established in 1974. Mullets migrate in schools from their feeding grounds in inshore waters to the open sea for spawning[11]. Estuaries and coastal lagoons provide the necessary conditions for growth of juvenile fish during a large part of their life[11]. When juvenile fish mature, they migrate back to the sea for the spawning[12]. Mullets are of great economic importance; the demand for mullet roe in many parts of the world has grown considerably in recent decades and elevated the status of grey mullets to be being called "grey gold" by fishermen[12]. Mullet farming has been practiced worldwide for centuries, especially in the Mediterranean. Aquaculture of grey mullets is based on the capture of fry and juveniles in their natural habitats[11,12]. *Such type of extensive aquaculture makes highly important to acquire knowledge about fish dispersal in the regions with appropriate opportunities for capture and epidemiological conditions for lagoon fish culture. The present project will provide such knowledge that will have a direct application in the aquaculture.*

Objectives and overview of the action

This project aims to investigate the effects of the Banyuls-Cerbère protected area on the preservation of the taxonomic and molecular diversity of marine parasites, and to perform assessment of the stock recovery of grey mullets in the open Mediterranean areas. We will address the lack of knowledge about parasite communities and host-parasite relationships in MPA, to maintain a broad ecosystem-based framework for management of marine biological resources. Taking as basis epidemiologic and demographic indicators, the project will generate estimation of the fishing rate that permit an efficient fish stock rebuilding.

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[7] Kingsolver JG, Hoekstra HE, Hoekstra JM, Berrigan D et al. (2001) The strength of phenotypic selection in natural populations. Amer.Nat., 157, 245–261

[8] Halpern BS et al (2010) Spillover from marine reserves and the replenishment of fish stocks. Environ Conserv, 36(4), 268-276

[9] Baldwin RE, Banks MA, Jacobson KC (2012) Integrating fish and parasite data as a holistic solution for identifying the elusive stock structure of Pacific sardines (*Sardinops sagax*) Reviews In Fish Biology And Fisheries, 22, 137-156.

[10] Blasco-Costa I, Waters J, Poulin R (2012) Swimming against the current: genetic structure, host mobility and the drift paradox in trematode parasites. Mol Ecol. 21: 207–217

[11] Saleh M (2008) Capture-based aquaculture of mullets in Egypt. In Lovatelli A and PF Holthus (eds). Capture-based aquaculture. Global overview. FAO Fisheries Technical Paper. No. 508. Rome, FAO, 109–126.

[12] Whitfield AK, Panfili J, Durand JD (2012) A global review of the cosmopolitan flathead mullet *Mugil cephalus* Linnaeus 1758 (Teleostei: Mugilidae), with emphasis on the biology, genetics, ecology and fisheries aspects of this apparent species complex. Rev Fish Biol Fisher, 22, 641-81.

The ***objectives*** of the project are: i) qualitative and quantitative characterization of grey mullet populations; ii) inventory of parasite faunas from different localities; iii) assessment of importance of the reserve effect for genetic conservation of parasites; iv) evaluation of the usefulness of parasite population genetic structure as biological tags in marine life conservation; v) comparative quantitative assessment of the temporal and spatial patterns of variations in the diversity and abundance of fish parasite communities; vi) identification of key species complexes within fish parasite communities, which can be used as fishery response indicators, on the basis of the quantitative relationship between host-parasite encounter and specificity filters; vii) evaluation of the parasite effect on the fitness and the survival rate of grey mullets along the spatial scale of the anthropogenic effect; viii) development of a predictive model to investigate equilibrium yield and parasite load inside and outside the reserve, to propose guidelines for management to control fish stocks, while considering local settings; ix) disseminate project findings on local, regional and national levels through a website, workshop, symposiums/conferences and publications.

Research methodology and approach

This section provides a description of the principal methodology emphasizing following approaches.

(i) *Individual-centred approach*. It will be carried out through fish and parasite surveys focussed on stocks and population of grey mullet, and infra-community and component communities of parasites in each host individual; exact parasites identification; detailed study of the morphology, taxonomy and specificity of parasites. Due to the obvious complexity of studying whole compound communities, the vast majority of parasite investigations have been carried out at the infra and component-population/community levels. The present study will be no exception since most analyses are performed at these two scales.

(ii) *Quantitative approach*. Quantitative assessment of parasites will include: analysis of parasite populations and communities and their dispersion patterns in host individuals and populations, statistical hypothesis testing based on quantitative replicated measurements. In the case of parasites, individual hosts will be treated as replicate habitats. Parasite species richness, abundance, prevalence and aggregation will be measured for each host-parasite association.

(iii) *Comparative approach*. Integrated, multi-species, ecosystem-level approach will be applied to explore the relationships between various ecosystem components and parasite community structure and dynamics to understand the ecology and potential population-level effects of MPA in the Mediterranean. A factorial sampling design will be adopted in this project allowing a comparative analysis of parasite community composition, aggregation and transmission dynamics with respect to geographical, environmental and time scales. The main hypothesis, which will be tested here, is that if there is an effect from protection, it will be reflected on parasite diversity, abundance, distribution and their population genetic structure. The effect of other putative explanatory variables, such as season, year, host population density, host age and geographic locality will be controlled.

(iv) *Age approach.* Tracking parasite dynamics over fish growth beginning from the first days of post-settlement will provide a real chance to assess the influence of the parasitic infection on the host mortality range and to compare fish survival rate between protected and open-access areas. In this study direct and indirect approaches will be adopted: i) the repeatable observations of parasite abundance, prevalence and aggregation on the same host population; and ii) Crofton's truncation technique[13] that is based on the comparative analysis of the tail of the observed distribution to that of the distribution predicted from the first few points of the data. Crofton claimed that there are usually fewer heavily infected hosts in wild populations than would be predicted. An explanation for this phenomenon is that heavily infected hosts are more predisposed to mortality.

(v) *Holistic multidisciplinary approach*. Parasite structure, abundance and haplotype diversity as biological tags will be considered to identify fish spillover. Integrating data from parasite infra-communities and haplotype population structure will provide a deeper insight into fish population structuring. The different levels of sensitivity of each dataset will bring different levels of information allowing the use of a holistic approach. The rationale of the genetic approach based on the haplotype structure of parasite populations is that it should identify host stock structure or host migration patterns more accurately than classic parasite abundance measurements[14,15].

(vi) *Forecasting approach.* A predictive model of host-parasite relationships will be developed with Generalized Additive Models (GAM). The main advantage of GAMs is that they allow to model both linear and nonlinear interactions between the dependent variable and the sets of predictors. Preliminary analysis and model selection have shown that the negative binomial distribution is the most appropriate for these counts (Shvydka, Sarabeev, Cadarso-Suarez, 2017). The forecasting approach opens perspective to move from consequence-oriented methods in epidemiology and fish management to examining factors promoting resource depletion. The wealth of data that will be gathered at different hierarchical levels in environmental and spatio-temporal framework holds real promise to identify the determinants of community structure of parasites in mullet stocks for generalisations of heuristic value, assessing the importance of resource partitioning by parasites in both time and space.

Originality and innovative aspects of the research programme

This project proposes a new approach that relies on fish parasites, using their community structure and dynamics, to assess the conservation, spillover and gene flow within protected and open-access areas, in order to improve the management of fish stocks. Ecologists traditionally focus on the "big picture" of stocks and flows of mass and energy at the whole system level, while parasites are usually neglected in environmental studies[16]. By ignoring the conservation of parasites we also ignore the conservation of the majority

[13] Crofton HD (1971) A quantitative approach to parasitism. Parasitology, 62, 179-193.

[14] Marcogliese DJ, Jacobson KC (2015) Parasites as biological tags of marine, freshwater and anadromous fishes in North America from the tropics to the Arctic. Parasitology 142, 68–89

[15] Klapper R, Kochmann J, O'Hara RB, Karl H, Kuhn T (2016) Parasites as biological tags for stock discrimination of beaked redfish (*Sebastes mentella*): parasite infra-communities vs. limited resolution of cytochrome markers. PLoS ONE 11(4): e0153964.

[16] Gomez A, Nichols E (2013) Neglected wild life: parasitic biodiversity as a conservation target. International Journal for Parasitology: Parasites and Wildlife, 2, 222–227.

of life on Earth, as parasitism represents the most common lifestyle: animal, unicellular and multicellular plant and fungi, virtually all free-living and even parasitic species are affected by parasites. We propose here to focus on upstream events and the impact of parasites, to gain a better understanding of the proximate and ultimate causes of ecosystem integrity. Our main goal is to move from a consequence-oriented method to an ecological framework, in which we will consider the multiple two-way interactions between the many host and parasite species interacting in an ecosystem. *This project integrates parasites in the management strategy of fishery resources to promote environmental conservation and sustainable use in an equitable way*.

The novelty of the study is to focus on fish early life-history stages, which are most vulnerable to parasites and predators. As fry or juvenile fish enter the inshore shallow areas, they come into increasing contact with parasites as they grow. Our last study of host-parasite relationships of metacercariae in mullet host suggests that metacercariae-associated mortality of juvenile fish caused by a single parasite species exceeds 50% of the recruitment during the first year of fish life (Sarabeev, 2015b). *Studies of fish early life-history stage will provide critical information on the effects of infectious disease on recruitment rate and fish survival probability*.

Moreover, the novelty of the present project lies in the application of a comparative approach at two hierarchical community levels (parasite infra- and component communities) and several nested scales along a spatio-temporal gradient. Major contributions have recently been made in macroecology and community ecology by adopting this comparative approach[17]. *The present project will apply such comparative approach to study parasite dynamics and community structure in protected and unprotected ecosystems*.

The originality of the project is the application of parasite infra-community and genetic data to assess the importance of MPA for conservation issues. Currently, there is no clear technique or set of techniques for assessing conservation, spillover and gene flow within MPA and neighbouring areas[9], as methodologies have not been yet developed, which would allow the complex estimation of objective factors influencing the ecosystem functionality at different hierarchical levels. This research will investigate the dynamics and interactions in a marine vertebrate host-parasite system and estimate the effect of the protected area on fish conservation and spillover using parasite communities and their genetic structure as fishery response indicators.

The best career possibilities for the experienced researcher and new collaboration opportunities for the host organisation that opens up the research

The financial support offered by MC IF will provide the required time and financial resources for the proposed research. The project will also be used to popularize the research results of the *Experienced Researcher* (*ER*) through presentations in international symposiums, while such financial support is very difficult to obtain in Ukraine because no Home University nor the Ministry of Education and Science of Ukraine (MESU) support such activity. The time and efforts of the *ER* will be devoted to the scientific work and training during the two years of the project to obtain new knowledge and specific experience, while in his home institution the main working activity will be dedicated to teaching with 600 academic hours per year. A high academic load and administrative duties are in conflict with the commitment required for a high level scientific activity. The list of per-reviewed publications of the *ER* is a good example of such conflict: between 2003 and 2007 and from 2012 to 2017, when his scientific activity was granted 12 and 11 papers were published, while only one paper was published between 2007 and 2011 when his main activity was administrative and teaching. *Therefore, the project will provide ideal conditions for the ER to publish a series of papers in top ranked journals and to communicate with academic and industrial partners.*

The *ER* had a couple of postdoc positions with his faculty position in the Home University (ZNU) beginning from 2001, when thanks to the INTAS sponsored fellowships he had a first opportunity to develop international collaborations. This short termed (2.5 months) international secondment has resulted in the establishment of a long term collaboration with a research team from the University of Valencia that is currently continuing (e.g. Sarabeev et al., 2013; Rodríguez-González et al., 2016). Great efforts were made to accumulate parasite data for further ecological analysis and to solve taxonomic and faunistic problems with respect to grey mullets parasites across the Mediterranean. In 2012 thanks to support of the MESU and collaboration with Serge Morand, the *ER* had obtained new expertise in area of parasitological data analysis with the application of aggregation indices (Sarabeev et al., 2017a,b). During the stay of the *ER* in Université Montpellier he had the first collaborative contact with Yves Desdevises that resulted in the publication of a paper exploring phylogenetic relationships among mullet monogeneans. Therefore, the present project is a logical follow up of the previous work of the *ER*. It will also provide a good opportunity for the *ER* to gain new knowledge and experience in molecular biology applied to parasitic organisms, the inventory of fish populations and the fishery management in the Mediterranean.

Host-parasite relationships undergo dynamic process in time and space, and so there is still a pressing need to regularly update the information on hosts and their parasites, their biology and relations with local communities, spread, and impacts on environment and economy. Based on the multidisciplinary approach developed during this project, that implies the use of parasites infra-community variability and genetic markers as integrated suite of indicators to evaluate effect of MPA on local communities, this work will combine the efforts of the invited *ER* and the Host Institution (*HI*) to increase our understanding of the effect of protection on local host and parasite communities. This will provide new knowledge about the various components of the marine ecosystem within the MPA and promote future actions through creating a *long-term collaboration* between the applicant and the participating organizations and other regions in the EU. The project will be focused on only one host species of grey mullets to highlight the usefulness of our holistic approach to assess the effect of MPA on local communities. We believe that after this project, our research should continue to be refined to a wide range of free-living organisms in European waters. It is expected that the project will promote *international*

[17] Guégan J-F, Constantin de Magny G (2007) Epidemiology in a changing world: The need for a bigger picture! In: Tibayrenc M (Eds.) Encyclopedia of Infectious Diseases: Modern Approaches. Wiley & Sons Ltd., Chichester, USA.

collaboration between both the host and home institutions, as well as partner organizations, through research, knowledge and expertise transfer. The joint project, with the collaboration of participated organizations is planned to take part in Horizon-2020 projects in sections "Aquatic Resources" and "Sustainably exploiting the diversity of marine life".

1.2 Clarity and quality of the training and the two way transfer of knowledge

Over the last years, the studies of the *ER* have focused on the classical taxonomy of mullets parasites in the Mediterranean (Sarabeev, Balbuena, 2003; Sarabeev, Balbuena, 2004a,b; Sarabeev et al., 2005; Rubtsova et al., 2006; Balbuena et al., 2006; Blasco et al., 2006; Holzer et al., 2006; Yurakhno et al., 2007; Sarabeev et al., 2013; Tkach et al., 2014), and the characterisation of parasite species using molecular data and phylogenetic approaches (Holzer et al., 2006; Yurakhno et al., 2007; Blasco-Costa et al., 2012; Sarabeev and Desdevises, 2014), modelling of parasite infection parameters with the application of ANNs (Sarabeev, Tkach, 2010) and GAMs (Shvydka et al., 2017), quantitative comparison of parasite species richness, abundance and aggregation patterns of native and invasive mullet hosts (Sarabeev, 2015a; Sarabeev et al., 2017a,b), and host parasite relationships of metacercaria and invasive grey mullet in the Azov Sea (Sarabeev, 2015b). Furthermore, one project was dedicated to the application of parasite communities of mullets as indicators of stock structure. More recently, the *ER* has been involved in the fishery industry through the execution of research-and-production projects in aquaculture. These experience and knowledge will help to advance the present project as it is logical continuation of previous work. At the same time, the *ER* lacks expertise and knowledge in non-capture methods of fish assessment and in molecular data acquisition and analysis. The planned mobility should fill a gap in such knowledge. *The core idea of the project is to couple the expertise of the ER in parasite taxonomy, ecology, host-parasite relationships on one hand, and the knowledge of participating organizations in management of wild fish population and in molecular biology and genetics on another hand, to contribute to the understanding of the effects of protected areas on parasite communities and fish populations in the Mediterranean areas. The project is concerned with transferring knowledge into Europe on the sharing and application of several methodological approaches described in section 1.1 being developed by a synergic effort of the academic staff of the participating organizations and the ER.* The *ER* will bring specialized skills and knowledge related to the new area of expertise in ecological parasitology being developed by the *HI*. Studies of host-parasite relationships and the development of modelling approach will be highly valuable for the staff of participating organizations as it will help to understand the effect of conservation on marine organisms, for a wide range of host-parasite systems. The invited *ER* is a top specialist in the field of mullet fish parasites, he has wide experience in the taxonomy of all groups of mullet parasites, including both the protozoans and metazoans. This will open for the *HI* a new research line focusing on parasitological studies of Mediterranean grey mullet parasites. Last but not least, in view of the variety of challenging research objectives and the wealth of analytical tools and techniques employed, this proposal has a high training potential. Thus the present project provides an excellent opportunity to carry out both the habilitation thesis of the ER and PhD thesis of the *HI* student.

1.3 Quality of the supervision and the hosting arrangements

The Supervisor of this project will be Yves Desdevises, Professor at the UPMC and deputy director of the OOB. He has a wide experience in the study of host-parasite interactions, in particular using molecular tools, starting from his PhD defended in 2001, on Mediterranean fish parasites. He has worked on a variety of host parasite systems, most of them marine, and especially parasites of fish (e.g. Sasal et al. 1998, Simkova et al. 2000, 2001, 2002, Desdevises et al. 2000, Desdevises 2001, 2006, 2007, Kaci-Chaouch et al. 2008, Poisot et al. 2011). He published a number of studies on fish parasites from the Mediterranean (e.g. Desdevises 2001, 2002, Poisot and Desdevises 2010, Mladineo et al. 2013, Sarabeev and Desdevises 2014) including studies in marine protected area (Sasal et al. 2004, Tessier et al. 2010). He has been co-PI of a Hubert Curien Partnership (2 years, 2009-2010) with Croatia (Cogito project) to assess the transfer of monogenean parasites between wild and cage-reared sparid fish in the Mediterranean using molecular tools. He has international collaborations with Czech Republic (group of Milan Gelnar, Masaryk University, Brno), Tunisia (Lassad Neifar, University of Sfax), Canada (Pierre Legendre and Timothée Poisot, University of Montreal), as well as national collaborations (University of Montpellier: Emmanuel Jousselin, Serge Morand; University of Perpignan: Olivier Verneau). He was promoted full professor in 2014, and is the deputy director of the OOB since 2011. He also is a member of the Scientific Committee of the NMRCB.

The OOB is part of the new EMBRC research network of European marine stations (http://www.embrc.eu), a new European Research Infrastructure Consortium (ERIC). As such, it offers up-to-date facilities to researchers from various countries. The researchers coming in Banyuls then interact with local researchers to implement new collaborations beneficial to all parts involved. The *ER* will interact with the local services in charge of animal collection and ecosystem preservation (CRBM, Centre de Ressources Biologiques Marines, *Center of Marine Biological Resources*) that will use his data to refine its inventory of the teleost fauna within the local environment. The hosting group is the new team led by Yves Desdevises, *Marine Interactions – Evolution and Adaptation* (INTERMED), within the *Integrative Biology of Marine Organisms* research unit. INTERMED focuses on different kinds of marine symbiotic associations (in particular fish and ectoparasites) using molecular biology to address evolutionary and environmental question. These skills will clearly benefit to the *ER*, who will in turn bring to the hosting team his expertise in fish and parasite taxonomy that is greatly in need in the INTERMED team where such knowledge is critical and difficult to find. The numerous national and international collaborations of the *ER* (see above) will allow the joint project to be integrated in a worldwide network.

The research activity described in the work plan (section 3.1) will be distributed among the staff of the *HI* and the partner organization: the supervisor will be engaged in the execution of work packages (WP) 4 and 5. The research will be done by applying knowledge exchange through training the ER in new laboratory and analytic methods of molecular studies. The WPs 1 and 2 of the work plan will be carried out with help of Jérôme Payrot, Responsible scientist of NMRCB, and Pascal Romans, head of the CRBM. Pascal is responsible for material sampling in the laboratory and experienced in techniques used for assessing fish assemblages. The

collaboration with the marine reserve staff will promote the implementation of the result as a management strategy of the protected area. A doctoral student of *HI* will be involved in the execution of WPs 2-7. The model of teaching through research will be applied here with respect to PhD student of *HI* and the staff of the marine reserve that will be done through the transfer of skills and knowledge from the ER. The WPs 2 and 4 that are related with laboratory studies will be supported by the technical staff of the *HI*. In addition, Julie Boeuf, in charge of the reception service of the OOB, will be responsible for providing all required permissions/licenses. The WPs 6-8 will be done in collaboration with Serge Morand (UM), one of the world's top-cited researcher in the ecology of parasitism.

1.4 Capacity of the researcher to reach and re-enforce a position of professional maturity in research

I am at stage of my career where science and gaining new knowledge is highly meaningful to me. I have spent 20 years in the field and feel as if I now have a lot to offer as a researcher. I believe that the multidisciplinary approach of the proposed project is the best way to evaluate the effects of protected areas on the marine communities and to assess the fishery benefits. I have acquired broad competence in laboratory and analytical works with parasites. I am familiar with protocols for parasitological surveys of vertebrate animals and methodologies of identification, fixation, concentration and preparation of parasites for identification. I have gained experience in dealing with the main taxonomic groups of fish parasites. I have shown capacity to learn a range of methods to investigate morphology, phylogeny, ecology, dynamics of parasites and host-parasite relationships such as descriptive statistics, frequency and aggregation analyses, discriminant, cluster and principal component analyses, non-metric multidimensional scaling, regression analysis, generalised additive models, ANOVA, ANCOVA, ROC-curves, rarefaction curves, nonparametric richness estimator Jackknife, Taylor's law and negative binominal distribution models, Crofton's truncation technique, GAMs, self-organizing maps (SOM) and back propagation (BP) algorithms of ANNs, cladistics and some others. I am familiar with a number of software packages for data analysis such as EXCEL, SPSS, PAST, MatLab, EstiMateS, PAUP, some packages of R and Mesquite. Practically all my publications contain new scientific information grounded on rich materials and a substantial analysis of field data. One of the advantages of this scientific work is application of the modern tools of numerical analysis that are expressed through publications.

I studied German in school, and I have achieved competence in 3 other European languages: English as first foreign language, Spanish (A2) and French (B1). As Banyuls is situated on the French border with Spain, the language barrier should not represent any problem. I have developed several international scientific collaborations with numerous academic partners: University of Valencia, Polytechnic University of Valencia, University Santiago de Compostela, Spain; University of Montpellier, University Pierre and Marie Curie, University of Maine, France; Institute of Parasitology in Warszawa, Poland; University of Ostrava, Czech Republic; and University of British Columbia, Canada.

I was the first to initiate systematic, fundamental and multifaceted studies of parasitological aspects of the introduced mullet host, *Liza haematocheilus*. A large part of my research addressed questions associated with the taxonomic, faunistic and ecological relationships between parasites and the introduced host. Our recent studies were devoted to the comparative quantitative analysis of parasite communities in native and invasive populations of grey mullets. These studies had important theoretical implications that may explain the success of invasive species in ecosystems (Sarabeev et al., 2017a). At the same time, native parasite species, forming new host-parasite system, can substantially affect the population of the invasive host, especially the fish yearly life-history stages (Sarabeev, 2015b). An important practical implication was derived from the results of our study: i) as parasite-related mortality was observed among high yielding stocks of *L. haematocheilus*, there is some reason to catch juvenile fish from wild population for their further growth in aquaculture; ii) as the metacercaria infection rapidly increased in zero year old juveniles, those should be removed from the natural environment for aquaculture growing at the early juvenile age, preferably in summer (Sarabeev, Domnich, 2000; Sarabeev, 2015b). Furthermore, I was the first to apply SOM and BP algorithms of ANNs to study host-parasite relationships and parasite dynamics (Rubtsova, Sarabeev et al., 2006; Sarabeev and Tkach, 2010).

I have developed my own research ideas, that were original and substantive enough for acceptance by the academic and industry communities in the form of peer-reviewed publications in ranked journals (including those from Q1 list), supported fellowships, research and research-and-production projects. I have organized 32 field expeditions to sample fish and parasites across Azov-Black Sea localities between 1997 and 2014, and more than 10 across the NW Mediterranean and the NE Atlantic between 2001 and 2016, where I have gained a strong expertise in field work and sample collection. I coordinated the work of 2-6 people for a period of 1-4 weeks in each expedition. Beginning from 2008 and up to the secondments to the University of Santiago de Compostela in 2016/2017, I combined teaching with administrative work as the head of the Regional Study-Research-Production Centre of "Ecology" in ZNU. I coordinated 4 laboratories and managed 18 people. Therefore, my capacity to become an independent and leading researcher in EU space is clearly established.

2. Impact

2.1 *Enhancing the potential and future career prospects of the Researcher*

The MC fellowship will lead the *ER* to acquire skills in a wide range of techniques for in depth studies of parasites and their hosts. In particular, he will gain new knowledge in molecular, ecological and epidemiological techniques and *there are few scientists with this array of skills in this domain in Europe*. This corresponds to a current demand to assess the effects of MPAs on diversity and richness of marine community and expected fishery benefits. In addition, this should help the *ER to find a permanent position* as research *scientist in the academic or industry sector of the European area*.

Moreover, the project will supply a substantial field and published material for the *habilitation thesis* of the *ER*. In this thesis, the taxonomic and faunistic chapters are completely published (11 papers) and prepared, while the next two chapters, one on parasite phylogeny and genetics (2 papers published), another on parasite ecology and host-parasite relationships (5 papers published), are in development. *Therefore, one of the expected results of the project is the preparation of the two chapters to complete the habilitation*

thesis of the ER. This highest academic qualification that can be obtained by the *ER* soon after the project completion, will give him the possibility *to become an independent researcher* and to set up his own group. Being a researcher in Ukraine is very difficult due to the latest events in the east of the country and the *ER* is striving to internationalize his career. Therefore, this project will offer the *ER* perfect opportunity to obtain new expertise and knowledge, to become a leading and independent researcher.

2.2 Results dissemination

The project results can contribute substantially to a number of disciplines such as community ecology, fisheries, conservation biology, epidemiology and molecular biology. By focusing on parasite communities of mullets at different scales of organization, from host individual to locality, along spatial and temporal scales considering anthropogenic effects, this work will provide new valuable information about how local, regional, temporal and anthropogenic factors define naturally occurring species assemblages, which is one of the current hot topics in community ecology[18]. The present study can also be useful to design environmental monitoring policies of marine protected and open-access areas in the Mediterranean.

The dissemination of the results will involve the distribution of: i) reports on scientific meetings and publications in refereed journals to communicate with the academic community; i) information in a user-friendly format for the general public by means of web-based resources, flyers, seminars, communication in the Biodiversarium and the final workshop. Preliminary and first-hand results will be presented at relevant scientific meetings and symposia: International Symposium on Fish Parasites in Brisbane, Australia in 2019; Annual European Marine Biology Symposium in 2019; 13th European Multicolloquium of Parasitology, in Belgrade, Serbia in August 2020. Internet-based information will be provided and a web portal will be set up to publicise the project, its objectives and main results. Flyers will be distributed among visitors of the marine reserve, participants of workshop and students of *HI*. The final 2.5 days workshop is planned on the 23rd month. The aims of this workshop are to bring together researchers, marine practitioners and decision makers to share the theoretical and practical knowledge obtained during the project, to highlight the role of parasites in MPAs and to identify the best practices, particularly in terms of biodiversity conservation and fish stock management. The workshop will be organized in the OOB; the direction of the *HI*, supervisor, key persons of partner organization and the *ER* will act together as scientific committee; the mass media will be invited for this meeting to popularize the sustainability in environmental resource management. In addition, printed information such as reports, flyers and posters, about intermediate and final outcomes of the project will be distributed among members of the Scientific Committee of the NMRCB and the Ministry of Agriculture, Agrifood and Forestry of France.

2.3. Communication: communication will include following formats: reports on symposia, seminars and workshop for academic, industrial communities and public; at least 5 scientific papers in peer-reviewed top ranking journals; flyers, posters, PowerPoint presentations and workshop proceedings will be distributed through the web site and scientific meetings; a web site with the project information, timetable, progress report of results and feedback section. The web site will be created during the first three months of the project and will be maintained by professionals of *HI* during the execution of the project and after its end. In particular, the outcomes of the project will be presented to the public in the new scientific popularization centre of the OOB, the Biodiversarium (http://www.biodiversarium.fr). The best reports, made in the final workshop will be published in a special issue of the scientific journal Vie et Milieu, which will be dedicated to management and conservation practices in MPAs.

3. Implementation

3.1 The work plan

Work package (WP) 1. Estimation of grey mullets assemblage and density in protected and open-access areas. Substantial differences in fish host density between protected and open-access waters are expected due to fishery pressure in unprotected areas. The estimation of fish density and assemblage will be obtained using underwater visual census (UVC) supplied by video recording. Furthermore, Experimental catch will be carried out after visual observation of fish, with a fry dragnet. Adult fish will be collected by spear-fishing or obtained in local fish markets. Fish assemblages are being studied in the Banyuls-Cerbere MPA[5] since the first survey by Bell[19] via UVC methods. The grey mullet community in the NW Mediterranean counts five species. The most prevalent species of grey mullets in the studied areas will be selected as the object of the present study after the first sample to save time and efforts required to collect and analyse samples and to meet ethical requirements concerning animal research. Sampled fish will be further subjected to meristic, parasitological and molecular studies that guaranty an optimal use of the biological material.

Milestone 1. Grey mullets assemblage and density estimated in the studied areas. *Objective 1 achieved. Deliverable 1.* Database with biological information of fish species composition and density.

WP 2. Sampling of fish and parasites. This project will follow a nested sampling design in order to analyse parasite community composition and abundance from grey mullets with respect to geographical location and time. Grey mullet individuals will be obtained in coastal waters at three Mediterranean localities: off Banyuls-Cerbère, France (MPA), off Cap de Creus (≈25 km from the MPA) and lagoons on delta of the Ebro, Spain (≈300 km from the MPA). The selected localities differ in fishing pressure on grey mullet stocks. In Southern France these fish traditionally are out of commercial fishing interest (personal comments of local fishermen). This fact together with the protection regime in the Banyuls-Cerbère MPA is a guarantee of minimal anthropogenic impact on local mullet stocks. In the Ebro lagoons mullets prevail in catches since 1966, but the production of finfish is reported to have declined there since 1970 due to overfishing and pollution[20]. In addition, seasonal samplings of adult fish from the Ebro lagoons will offer the opportunity to

[18] Gaston KJ, Blackburn TM (2000) Pattern and process in macroecology. Blackwell Science, Oxford.
[19] Bell J.D. (1983) Effects of depth and marine reserve fishing restrictions on the structure of a rocky reef fish assemblage in the northwestern Mediterranean Sea. Journal of Applied Ecology, 20, 357-369.
[20] Dill WA (1990) Inland fisheries of Europe. EIFAC. Technical Paper. No. 52. Rome, FAO. 1990. 471 p.

compare this newly obtained material with that sampled in 2004-2005 by INTAS project #03-51-5998 to test time effect on parasite communities. The coastal zone of the Cap de Creus is similar to the Banyuls-Cerbère area, as a rocky-shore locality, but with artisanal coastal fishery.

Grey mullets first enter coastal waters when individuals are between 10 and 30 mm of standard length, then approximately 2-4 months old[12]. Fry and juvenile mostly appear in coastal waters in winter and spring depending on fish species, environmental differences and distance from the spawning area. Fry and juvenile grey mullets of zero-year old will be sampled for analysis from the MPA in the days following their entrance in coastal waters: first several days, 30, 60, 120, 180, 270 and 360 days, whereas from open-access areas those will be sampled once per season on the same dates as fish from MPA, providing 4 additional samples from each locality. Adult fish will be sampled twice at each locality in spring and autumn. At least 25-40 fish individuals will be collected per sample. The sampling plan will then include 15 distinct samples to assess fish yearly life-history stages and six samples of adult fish that will count about 650 fish individuals in total.

All fish will be transported to the laboratory of the OOB, where they will be surveyed for parasites, according to a standardized sampling protocol. Each fish will be weighed and measured, then dissected under a stereomicroscope. The wet liver weight will be recorded to compute the hepatosomatic index (HSI) as descriptor of the physiological condition of the fish. All organs will be inspected for parasites. Total collection and counting of parasites will be performed. Parasite specimens for morphological identification and molecular studies will be fixed and preserved in 70 % ethanol and preserved in a fridge.

Milestone 2. All fish required sampled. *Deliverable 2.* Fish biometric database.

WP 3. Inventory of the parasite faunas and analysis of species richness from the protected and open-access localities. Parasites will be prepared for morphological study according to their taxonomic group (staining, clearing, mounting, etc.), following well established protocols[21]. A thorough study of their morphology will be carried out in order to achieve a reliable identification of each specimen. The parasites identification will allow us to define the composition of parasite community of each fish individual and sample.

Following Sarabeev (2015a) we will use two measures to characterize the size and diversity of parasite assemblages: i) parasite species richness (PSR) at the local and total levels, which defines the PSR of a sample or a locality; ii) individual parasite species richness (IPSR) and its mean (MIPSR), including uninfected individuals, in the sample. The working hypothesis of the study is that species richness is higher in the MPA and its neighbouring areas when compared to intensively fished area. Sample-based rarefaction curves supplied by 95 % confidence intervals will be used to compare PSR from different fish samples. The non-parametric richness estimator Jackknife (first order) will be calculated to deduce the total species richness in each parasite community. Both the rarefaction curves and Jack 1 will be obtained using EstiMateS (http://viceroy.eeb.uconn.edu/estimates/). The effect of fishery on MIPSR will be tested using ANOVA. The pooled dataset gathered form the new samples and those obtained in 2004-2005 will be used for this objective. Only parasite data from adult fish individuals will be used. The overall dataset will include parasite surveys from mullets from Santa Pola Salt Marshes, Santa Pola Gulf, Ebro Delta (see details in Sarabeev 2015a), coastal waters off Cap de Creus and off Banyuls-Cerbère. The time effect on parasite richness will be controlled for.

Milestone 3.1. Successful identification of the parasites and major taxonomic questions solved. *3.2.* Parasite species richness analysed. *Objective 2 achieved. Deliverable 3.1.* Host-parasite database with parasite infra-population size and species composition. *3.2.* Publication on comparative analysis of parasite species richness between protected and open-access areas.

WP 4. Sequence acquisition. Only the best characterized host-parasite associations will be used. Parasite species from the genera *Ligophorus*, *Neoechinorhynchus* and *Rhipidocotyle* are good potential candidates for this study. Total genomic DNA from single parasites will be extracted using commercial (Qiagen) DNA extraction kits. The partial ITS2 rDNA and the mtDNA cytochrome oxidase subunit 1 (COI1) and 2 (COI2) will be targeted for amplification; they have proven useful in population genetic studies of helminth parasites[14,22]. Universal worm primers (e.g.[23]) or specific ones developed from sequences of congeneric species in GenBank will be used for the amplification reaction. Samples of purified PCR fragments mixed with primers will be labelled and sequenced using the Bio2Mar technological platform of OOB (http://bio2mar.obs-banyuls.fr). Twenty-five sequences per locality for each species will be acquired, which will guaranty an accurate estimation of the population-level molecular diversity[24].

Milestone 4. Molecular data for subsequent population structure analysis obtained. *Deliverable 4.* Database with sequences.

WP 5. Exploring the effect of MPA on parasite genetic diversity and gene flow. Haplotype and nucleotide diversities among populations of the selected species will be compared between protected and open-access areas. The working hypothesis is that the haplotype diversity of selected parasite species from the Banyuls-Cerbère MPA is higher than in the Ebro lagoons which are currently suffering fishery and anthropogenic-related damage of ecosystem, whereas in the neighbouring waters, of the MPA (Cap Creus) the diversity is expected to be equal due to spillover. Analyses of the rDNA and the mtDNA sequences will be performed with DNAsp (http://www.ub.edu/dnasp) to calculate the following statistics: number of unique haplotypes, haplotype diversity (h), nucleotide diversity (π). Tajima's D and Fu's F will be executed to test for selective neutrality and demographic processes. Pairwise and overall

[21] Dailey MD (1996) Meyer, Olsen & Schmidt's essentials of parasitology. Ed. M.J. Kemp. Wm. C. Brown Publishers.

[22] Bazsalovicsová E, Králová-Hromadová I, Štefka J et al. (2011) Population study of Atractolytocestus huronensis (Cestoda: Caryophyllidea), an invasive parasite of common carp introduced to Europe: mitochondrial cox1 haplotypes and intragenomic ribosomal ITS2 variants. Parasitol. Res. 109, 125–131.

[23] Moszczynska, A., Locke SA, Mclaughlin JD, Marcogliese DJ, Crease TJ (2009) Development of primers for the mitochondrial cytochrome c oxidase I gene in digenetic trematodes (Platyhelminthes) illustrates the challenge of barcoding parasitic helminths. Mol. Ecol. Resour. 9, 75–82.

[24] Goodall-Copestake WP, Tarling GA, Murphy EJ (2012) On the comparison of population-level estimates of haplotype and nucleotide diversity: a case study using the gene cox1 in animals. Heredity (Edinb). 109, 50–56.

distances among haplotype sequences will be performed using MEGA (http://www.megasoftware.net). Analysis of molecular variances (AMOVA) will be conducted with permutations to test the within and between variation for the different localities. Pairwise genetic differentiation among localities will be estimated using the fixation index (F_{ST}). AMOVA, FST, Tajima's D and Fu's F will be estimated using Arlequin 3.5 (http://cmpg.unibe.ch/software/arlequin35). A median-joining haplotype network will be created for selected parasites species to investigate genetic relationships among haplotypes from different localities using NETWORK (http://www.fluxus-engineering.com/sharenet.htm).

Milestone 5.1. Effect of MPA on parasite haplotype diversity and gene flow assessed. *5.2.* Usefulness of parasites population genetic structure as biological tags for differentiation of the protected area evaluated. *Objective 3 and 4 achieved*. *Deliverable 5.* Publication on the effect of MPA on gene conservation and gene flow with data on the use of parasite population genetic structure for fish tagging in top ecology journals.

WP 6. Effect of season, habitat and locality on the structure and abundance of parasites. The analysis will be carried out under the working hypothesis that if there are distinctions in the fishery pressure on the local fish communities, and changes in the temporal and local host distributions, they will be reflected on the number of species of parasite communities and in their abundance and distribution among host individuals. The hypothesis will be tested in the same way in both adult and zero-year old juvenile fish. The mixed dataset from previous (2004-2005) and new surveys of parasites will be used for adult fish. Variations in parasite species community will be examined with the application of different methods in order to summarize and visualize the variability of the data and to find the best methods and key parasite species.

Partial linear regression will be used to evaluate the influence of season and protection on parasite species at the infra-community level. This method is appropriate because it affords estimating how much of the variation of the response variable can be attributed exclusively to one set of factors, once the effect of the other sets of factors have been taken into account and controlled for[25], assuming a linear relationship for the response variable across the factors. The response variables will be species richness and diversity, whereas explanatory variables will be allocated into three categories: environmental, spatial and temporal factors[25]. The similarity in species composition between communities will be measured by Bray-Curtis indices. Non-metric multi-dimensional scaling (MDS) ordination will be performed on the matrices of these indices in order to visualize the low-dimensional relationships among infracommunities and component communities. Then, hierarchical cluster analysis will be superimposed on the MDS plots to identify communities. Genuine clusters will be identified by permutation tests to determine whether each node of the dendrogram has a significant support. It is expected that clustering patterns of component communities will be congruent with fish age and/or locality, if these variables have important structuring effects. Analyses at the component community level will be carried out with PRIMER (http://www.primer-e.com), due to its ample assortment of multivariate procedures for analyzing species/samples abundance matrices. The component communities will be defined by season and locality. An Analysis of Similarities (ANOSIM) will be performed test the null hypothesis of no differences in component communities. A crossed layout will be adopted to take into account the nested nature of the sampling design. Special emphasis will be given on the selection of candidate parasites for use as biological tags of fish stock discrimination. MacKenzie and Abaunza[26] criteria for the selection of suitable parasites will be applied.

Milestone 6.1. Evaluation of effects of environmental and temporal variables on the structure and composition of the infracommunities and component communities. *Objectives 5 achieved. Milestone 6.2.* Identification of key species complexes within fish parasite communities, that can be used as fishery response indicators. *Objectives 6 achieved*. *Deliverable 6.* Publication reporting environmental, spatial and temporal effects in top ranking parasitology journals.

WP 7. Effect of fishery and parasites on the fitness and the survival rate of grey mullets. Size, weight and HSI will be used as estimates of the biometric and metabolic condition of mullets. The primary objective is to examine whether there is a difference between the effects of parasite infection in fish caught from protected and open-access areas. Differences in size, weight and HSI between the areas will be analysed with a general linear model (GLM). Log-transformed size, weight and HSI will be used as response variables, infection and areas will be used as fixed crossed factors with fish age as a covariate.

The impact of parasites on fry and juveniles of grey mullets from protected and open-access localities will be evaluated by comparing parasite loads of different fish age groups using three analytical techniques: i) comparison of parasite prevalence and abundance between different fish age groups, ii) comparison of parasite aggregation, iii) parasite frequencies analysis in fish using the negative binomial truncation technique developed by Crofton[13]. Only long-lived parasites such as encysted helminth larvae (e.g. metacercariae of *Phagicola* and *Rhipidocotyle*) can be used for this objective. Similar studies carried out by the *ER* have shown the usefulness of these techniques in the study of host-parasite relationships (Sarabeev, 2015b).

Milestone 7.1. Estimation of the effect of parasites on the fitness and the survival rate of grey mullets. *Milestone 7.2.* Availability of terms for juvenile capture and epidemiological conditions of grey mullets for lagoon aquaculture. *Objective 7 achieved*. *Deliverable 7.* Publication on age related parasite dynamics in grey mullets and the effect of parasites on the fitness and the survival rate of fish.

WP 8. Model development. A simulation model parameterized with field data obtained in the first three WPs will be developed to predict the dynamics of selected parasite species load on fish stocks in the studied areas and to identify key factors affecting parasite infection parameters in mullet hosts. Current data suggest that the following parasite species are possible candidates for this part of the study: the monogeneans *Ligophorus* spp., the acanthocephalans *Neoechinorhynchus* spp., adult digeneans Haploporidae and

[25] Legendre P, Legendre L (1998) Numerical ecology (second edition). Elsevier Publishers, Amsterdam, Netherlands.
[26] MacKenzie K, Abaunza P. (1998) Parasites as biological tags for stock discrimination of marine fish: a guide to procedures and methods. FishRes., 1998, 38: 45–56

metacercariae of *Phagicola*. To investigate the effect of different covariates on the variable of interest, GAMs will be applied using the R package 'mgcv'[27]. Two types of responses will be considered, namely, parasite abundance and binary (prevalence) responses. The initial dataset will contain a large array of explanatory variables including five continuous (fish length, weight, fish density, average monthly water temperature and salinity) and five nominal (season, month, year habitat and collection locality). A preliminary work will be carried out to identify and remove some of the highly correlated (continuous) explanatory variables. A forward stepwise approach will be used to select the continuous and nominal explanatory variables. A set of models reflecting all the various combinations of potential covariates will be tested until only significant predictors will be left in the final model. Weakly significant variables will not be included as providing only a minimal improvement to the model. The models will be fitted using regression P-splines for the smooth terms. Each model fit will be assessed with respect to the level of deviance explained, Akaike's information criterion and by observing model residuals. The deviance explained by the final models and the deviance contribution of each predictor selected in the final models will be calculated. The deviance contribution of the predictors will be estimated based on the percent difference in explained deviance of the final models after deletion of one predictor at a time while keeping all the others (i.e. with replacement)[28].

Milestone 8.1. Predictive model and guidelines for controlling host-parasite relationships adapted to the local settings. *Milestone 8.2.* Improved methods to model parasite load on fish populations. *Objective 8 achieved*. *Deliverable 8.* Publication addressing the modelling of parasite load on host population under different environmental conditions.

WP 9. Communication, publication and dissemination of results.

Milestone 9. Communicate the results of the project according to Section 2 of the present proposal. *Objective 9 achieved*

Deliverable 9. Publications in peer-reviewed top journals in the disciplines of parasitology, ecology, evolution, environmental protection and fishery; information on the project outcomes through the web site, as well as on printed sources such as flyers, posters, presentations and reports.

Secondment. We consider that the three months secondment under the supervision of Serge Morand at the University of Montpellier is required mainly for the in depth and accurate analysis of ecological data.

3.2 Appropriateness of the allocation of tasks and resources

The present project is based on field surveys of wild fish and their parasites, which are scheduled on the first year. The field survey is a complex and time-consuming procedure that includes fish visual observation, catch, dissection, parasite sampling and counting, their fixation and cameral processing. In addition, all routine work with sequences acquisition is planned to be performed for the first 9 months. The technical staff and PhD student are expected to support general laboratory work, including DNA extraction, purification and amplification. Sequences will be obtained from the Bio2Mar platform. The work plan assumes acquisition of four databases (deliverables 1; 2; 3.1. and 4) up to the end of the first year; one publication in peer-reviewed journal to be prepared (deliverable 5) during the last quarter of the first year. The second year is dedicated to data analysis and redaction of peer-reviewed papers (deliverables 3.2.; 6; 7 and 8), results dissemination, and to complete the field survey if needed.

The institutional costs will be related to the sampling procedures and laboratory work, and most of the costs will be allocated to molecular studies. These costs are expected to be for the first year, while in the second year the financial efforts will be targeted on result dissemination via publications, workshop organization and participation in symposiums.

3.3 The management structure and procedures

Financial accounting will be processed through the Bursar's Office of UPMC (*Bureau Europe*: http://www.upmc.fr/fr/international/europe.html). The supervisor will be responsible for the overall management and supervision of the project and for distributions of reports to the MC fund. He will organize short weekly team meetings to set out project progress and plans. The investigations will be led by the *ER*, assisted by a doctoral student and a technician (both to be hired during the execution of the project). The ER will strongly interact with the hosting team in Banyuls, in particular the Supervisor, that will transfer him their knowledge and skills on molecular approaches to investigate parasite diversity. Frequent internal meetings will be organized to discuss the results and produce scientific papers in synergy. The *ER* will also have the opportunity to help the hosting team on their other research projects in the same scientific area, in which its skills will be of great added-value. The *ER* will be responsible for scientific progress of the project and production of reports. The number of persons involved is adequate to carry out the multiple tasks of the project. In particular, the participation of a technician and a doctoral student is fundamental to assist with fish and parasites samplings; DNA extraction and amplification. Secondment in University of Montpellier will be supervised by Dr. Serge Morand, Professor of AGIRS. He will coordinate studies on host-parasite relationships and the model development.

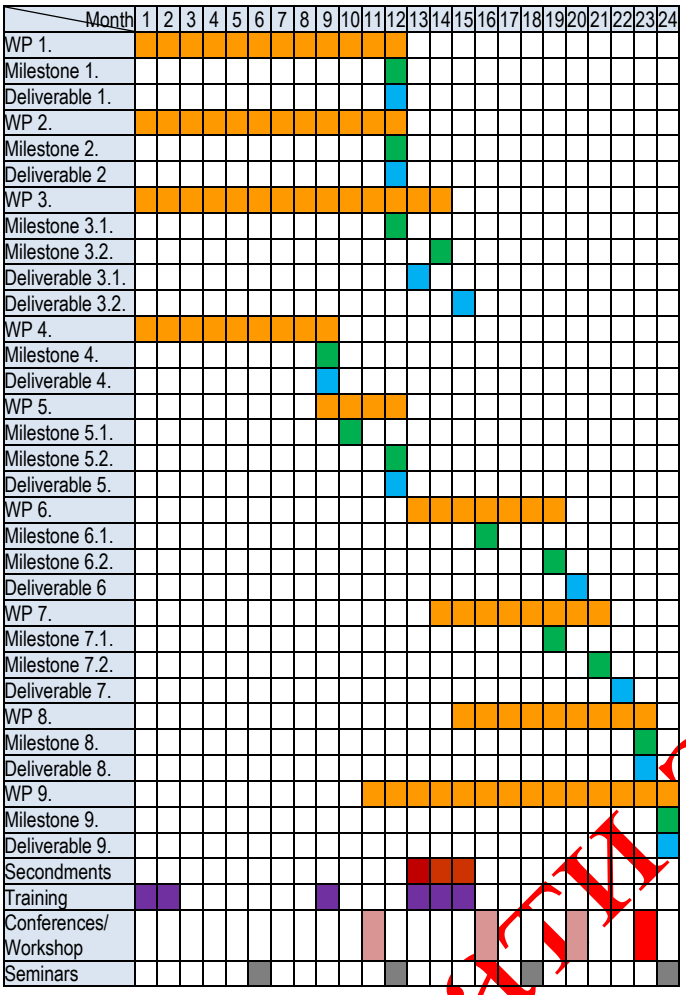
Within the first month of the project we will hold a Training and Coordination meeting. At this meeting, we will officially launch the project and establish initial good working procedures between staff of the *HI* and the invited *ER*. The periodical report meetings will be arranged on a formal basis twice a year and will be organized in the form of seminars. The fellow will supply a brief and concise written and verbal report on the research progress. These seminars will assess research progress against committed deliverables and milestones and, when necessary, redefine and agree on short and medium term objectives. A mid-term review of the project will be carried out at the beginning of the 12th month. The fellow will provide, for this review, a detailed written and verbal report of the progress of the research, with special emphasis on the achievement of the agreed deliverables and milestones. The verbal and written reports will be presented to the scientific committee of the OOB, and the written report will be sent to the European Commission. The

[27] Wood, S. (2016) Package 'mgcv'. http://cran.r-project.org/web/packages/mgcv/mgcv.pdf

[28] Llope M, Chan K-S, Ciannelli L, Reid PC, Stige LC, Stenseth NC (2009) Effects of environmental conditions on the seasonal distribution of phytoplankton biomass in the North Sea. Limnol. Oceanogr. 54, 512–524.

scientific committee will critically assess these written and verbal reports, and provide support and advice if the project is facing technical difficulties that hamper the delivery of expected results on time. The coordinator and fellow will, following critical appraisals, revise and amend, when necessary, the work plan and milestones to ensure the successful completion of the project. A final general review is planned on month 23 to assess the results of the project. The main objective of this last review will be to elaborate the strategies for releasing applicable outcomes of the research to managers.

Gantt Chart



The project success largely depends on a careful respect of the designed sampling plan (required sample size and time-frame) that can be exposed to natural and anthropogenic risks. However, these potential issues can be addressed by repeating the sampling procedure in the following several days or the missing sample can even be carried out in the next year. Additional sampling is possible because the project sampling plan is scheduled for the first year of the project execution.

A widely used technique of PCR-amplified marker genes will be used to obtain sequences of COX and ITS regions. While this approach is powerful, it has several technical limitations, which may distort the estimation of haplotype diversity and frequencies observed in the sequence dataset. Such limitations include choices relating to sample collection, sample storage and preservation, contamination, DNA extraction, amplifying primers, sequencing technology and read length. To reduce the impact of contaminants and other factors in sequence-based, low-biomass parasite studies, the following procedures will be applied: i) fixation and extraction of only maximally fresh material; ii) maximization of the starting sample biomass by applying cloning protocol[29]; iii) treatment of biological material, PCR and extraction kit reagents; iv) quantification of the negative controls and samples; v) replications and random processing of samples to avoid creating false patterns.

3.4 Appropriateness of the institutional environment

Beneficiary or HI: OOB of UPMC. The laboratory was created in 1881 in a location with exceptional marine biodiversity and well-situated for oceanographic studies. The laboratory employs ca. 180 staff members. The OOB possesses its own research and education buildings (currently displayed across 5 research and service units), experimental laboratories, a public and experimental aquarium and a very recent accommodation centre. Laboratories and climate control rooms with access to all basic research equipment are available, with technical support from the local staff. The institute provides access to library, computers and video equipment, and a database containing local environmental physico-chemical and biological descriptors is available. The OOB includes an imaging platform with a transmission electron microscope and a two-photon laser scanning confocal microscope, and a culture platform (new equipment) for mass production of microorganisms. The OOB possesses two vessels dedicated to research and diving activities. Access includes all on board research equipment, and technical support from vessel crew. The geographic location of the OOB will facilitate the logistics of the sampling process providing access to the protected locality, whereas the most distant open-access site is at less than four hours driving distance and the closest one at less than one hour. The OOB has a wide experience in the management of European research project, as being currently strongly involved in the ASSEMBLE initiative (http://www.assemblemarine.org) and the ERIC EMBRC-Europe (http://www.embrc.eu) mentioned above and in FP7 actions MAMBA (Marine Metagenomics for New Biotechnological Applications) and MicroAqua (Universal microarrays for the evaluation of fresh-water quality based on detection of pathogens and their toxins).

Role and Contribution: OOB will be responsible of project management, supervision of research progress and execution of WPs 1-4, sampling coordination, development of molecular tools, workshop organization and maintenance of web-based information.

Partner organizations: The NMRCB is fully equipped to carry out the monitoring of fish assemblage and population dynamics. The present project coincides with at least two main objectives of the NMRCB: scientific monitoring of marine organisms; preserving small, traditional fishing activities. This organization will be contributed to carry out WPs 1-2 and to exploit project results within the management plan of the reserve. The research activity of the AGIRS of UM focuses on the ecology and epidemiology of animal diseases. The AGIRS will coordinate the analysis of parasite communities, the assessment of the survival rate of grey mullets and model development (WPs 6-8).

[29] Leiro J, Iglesias R, Paramá A (2002) PCR detection of Tetramicra brevifilum (Microspora) infection in turbot (Scophthalmus maximus L.) musculature. Parasitol 124, 145–151