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FLAGSHIP

COMPLETE

BIOFOULING ASSESSMENT PROTOCOL FOR LEISURE BOATS AND MARINAS

COMPLETE WP 2.2

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CONTENT

INTRODUCTION

This protocol has been developed as a part of the COMPLETE-project (Completing management options in the Baltic Sea Region to reduce risk of invasive species introduction by shipping) funded by the Interreg Baltic Sea Region Programme. The aim of this protocol is to identify the potential risk of non-indigenous species (NIS) transfer by leisure boats and trailers in the Baltic Sea region. The sampling methods were tested in Finland during summer 2018, and the final protocol was developed according to the field-testing experience.

The protocol consists of the following sub tasks:

1. **Questionnaire**
2. **Sampling in marinas**
3. **Post season examination of fouling levels of leisure craft**

1. QUESTIONNAIRE

The questionnaire for leisure boaters in the Baltic Sea region (Appendix 1) will address such questions as the movement of leisure boats in the Baltic Sea region, anti-fouling method of choice, the use of trailer, cleaning procedures etc. The aim of the questionnaire is to obtain background information of the potential risk of transferring NIS by leisure boats and trailers via biofouling. The questionnaire is available online in respective language versions (link in Appendix 1), but it can also be printed out and answered as a paper version in boating events and marinas, for example.

2. SAMPLING IN MARINAS

Sampling in marinas by settling plates and scraping samples will address the question to what extent marinas represent a risk in introducing invasive species via leisure boats through biofouling in the Baltic Sea.

2.1 Material and methods

2.1.1 Location and subdivision of areas

The sampling should be conducted in a marina which is popular amongst boaters. The sampling should be carried out without disturbing the activity in the marina.

The sampling in a marina should be conducted in two subareas: 1) **inner marina** and 2) **outer marina**. The inner marina should be located close to the shoreline and sheltered by e.g. anthropogenic or natural structures, such as piers or banks. The outer marina should be located at the edge of a marina, further away from the shoreline, and it should be more exposed. The sampling spot in both subareas must be at least 1,3 m in depth to enable the sampling done properly. GPS location of each of the sampling site should be recorded by using a WGS84 coordinate system.

2.1.2 Settling plates

The settling plates (Figure 1) mimic hull surfaces to further indicate the potential risk of a non-indigenous species being spread through marinas. PVC is widely used as a settlement surface in biofouling studies and is therefore used as the material of the sampling plates in this protocol.

The methodology used in this protocol follows close to that of OSPAR/HELCOM port survey protocol (2015). The size of a settling plate should be 15.0 x 15.0 cm. The plates are attached to a polypropylene cord so that one plate set consists of two plates (Figure 2). The two plates should be attached to the cord at depths of 0.5 m and 1.0 m measured from the water surface. Each plate should have a hole drilled in the middle where the cord is attached. The hole should have a diameter of 0.5 cm and cord a diameter of 0.4 cm. To prevent the cord of being cut by the edges of the plate, a plastic tube or duct tape should be placed in between the cord and the plate. The plates should be secured at desired depths with knots or zip ties. The plates should be deployed horizontally to the sampling site since horizontally orientated plates have been observed to support a higher percentage cover of biofouling than vertical plates (Tait et al. 2016). One subarea should be equipped with three plate sets, in other words six plates in total per subarea.

A weight (e.g. a small brick) should be attached at the end of the cord to ensure that the set stays vertical. The plate sets should be attached to a buoyant wharf, berth or pier in a place where they will be safe from damage caused by passing boats. In case the plate sets are attached to a stationary pier, a buoy should also be attached to the set to secure that the plates remain submerged at desired depths.

Three settling plate sets should be deployed to each subarea in the beginning of the boating season. Since the growing season varies in length and timing in the Baltic Sea region, the sampling periods have been determined different for different areas. The length of the sampling season depends on in which basin the marina is located in. The Baltic Sea has been divided into 17 sub-basins by HELCOM (Subbasins 2018, HELCOM Monitoring and Assessment Strategy). For the use of this protocol these basins were grouped into three groups based on both salinity and surface water temperature averages during spring and fall since these factors are considered to be the most important ones affecting the fouling rates and species compositions in the Baltic Sea.

In this protocol, group A. includes the southwestern basins of the Baltic Sea, group B. includes the mid-basins and group C. includes the northern basins (Table 1). The sampling period for **group A. is 6 months** (suggested start at April until the end of September), **group B**. has a sampling period of **5 months** (suggested start in early May until the end of September) and **group C.** has a sampling period of **3 months** (suggested start in the beginning of June until the end of August).

The settling plate sets should be removed gradually in each subarea. The sampling period should be divided evenly into three shorter time periods. At the end of each shorter period one plate set should be removed in each subarea. The gradually performed removal provides information of species emergence and abundance during the boating season. Group A. should remove a plate set every eight weeks, group B. should remove a set every six or seven weeks and group C. should remove a set every four weeks (Table 1).

After the plates are removed, the fouling rates should be determined in relative abundance by placing a grid on the panel, dividing the area to four sub-quadrats each a size of 6.5 x 6.5 cm. The fouling rate of the panel is then analysed using a 5% interval (Dziubińska and Janas, 2007). If the percentual coverage exceeds 100 % because of organisms growing on top of each other, it should be noted when estimating the fouling rate.

The organisms on the plates should be identified with a microscope to species level or the lowest taxonomic level possible. The species on the plate should be listed and the individual abundances counted, at least for NIS. In case the native species' abundances are not counted, the most dominant species should be documented to control the type of the species assemblage.

The plates should be handled carefully to prevent the loss of organisms. In case an unknown species is found, it should be first photographed and then preserved in e.g. ethanol for further studies.

Figure 1. Settling plates.

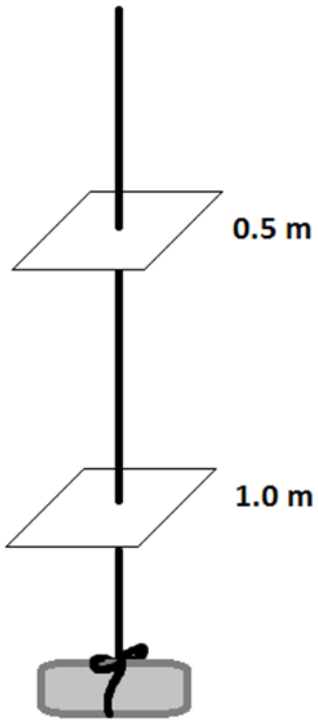

Figure 2. A settling plate set.

Table 1. The grouped division of the sub-basins in the Baltic Sea.

Group	HELCOM-ID	Name	Suggested sampling period	Suggested plate removal interval
A.	SEA-001	Kattegat	6 months (April-September)	8 weeks
	SEA-002	Great Belt		
	SEA-003	The Sound		
	SEA-004	Kiel Bay		
	SEA-005	Bay of Mecklenburg		
	SEA-006	Arkona Basin		
B.	SEA-007	Bornholm Basin	5 months (May-September)	6-7 weeks
	SEA-008	Gdansk Basin		
	SEA-009	Eastern Gotland Basin		
	SEA-010	Western Gotland Basin		
	SEA-011	Gulf of Riga		
	SEA-012	Northern Baltic Proper		
C.	SEA-013	Gulf of Finland	3 months (June-August)	4 weeks
	SEA-014	Åland Sea		
	SEA-015	Bothnian Sea		
	SEA-016	The Quark		
	SEA-017	Bothnian Bay		

2.1.3 Scraping samples

Since the plates provide a horizontally oriented surface for biofouling, additional scraping samples should be taken on vertically oriented berth surfaces or other structures present. Three scraping samples should be taken in each subarea when the settling plates are deployed and during each plate removal.

The scraping tool is a hand dredge (Figure 3). The mesh size of the net bag is 0.1 cm and the sharp edge of the dredge should be at least 10.0 cm wide. It is important to record the size of the surface sampled for future reference. When scraping, the sharp edge should be placed on the sampled surface in 1.0 m depth measured from the water surface and drawn upwards until it meets the surface. As the tool is drawn against the surface, the sharp edge scrapes the biofouling species and they fall into the net. Organisms fallen in the net should be identified to the lowest taxonomic level possible, listed and abundances counted with a microscope. If there are no structures present with a surface reaching 1.0 m depth, the sample is taken in the closest possible depth. In case an unknown species is found, it should first be photographed and then preserved in e.g. ethanol for further studies.

Figure 3. Scraping sample tool.

2.1.4 Salinity

The surface salinity should be recorded in each subarea when the plates are deployed and removed. Additional environmental information (such as: surface temperature, chlorophyll a, waves etc.) can be obtained e.g. through the EU Copernicus portal.

2.1.5 Optional enhancements for sampling in marinas

The methodology in this protocol is considered to represent the minimum requirements for monitoring marinas (in case of NIS being present and spread). To further increase accuracy and to better meet scientific integrity, additional methodology is presented here.

a) Additional settling plates can always be added to increase sample size to better meet requirements for statistical analysis. It is also recommended to add settling plates on different depths, *additional* to the two presented in this protocol (0.5 m and 1.0 m) if monitoring is conducted in marinas or harbours in deeper waters.

b) Replacing settling plate sets. When a settling plate set is removed, a new set can be deployed to increase sample size.

c) Sediment sampling is highly recommended as a complimentary methodology that will give wider knowledge of the area. If possible, the soft benthos could be sampled by using the OSPAR/HELCOM port survey protocol (2015) methods. Three samples per subarea should be taken mid-season.

3. POST SEASON EXAMINATION OF FOULING LEVELS OF LEISURE CRAFT

The importance of the task lies in mapping the overall risk of NIS introduction by examining the general fouling level of the vessel, photographing the fouled surfaces and sampling biofouling communities on the hull or niche areas of the vessel.

Optimally, most leisure boats are removed from the harbours and lifted out of the water after the season (at least in the northern parts of the Baltic Sea), which gives an opportunity to examine a good number of leisure boats in a more precise manner.

At its best, the post season examination should be conducted on leisure craft from marinas where settling plates were deployed to allow comparison between the two. To sample boats from a specific marina will more than often be challenging, since many popular marinas do not provide winter service and most boats are taken from the water elsewhere.

Further challenges for examination of leisure crafts consists of receiving permits to photographing and sampling from boat owners and personnel at the respective dockyard. Since boat owners are not obligated to participate, getting a good sample size might be challenging. The time for a thorough sampling might be restricted to only a few minutes because the companies managing boat lifting want to make their working time efficient. Also, one might more than often find it hard to sample hull surfaces, since the very common use of antifouling paints greatly reduces (or entirely blocks) biofouling. Therefore, recognizing unpainted structures and so-called niche areas, such as outboard motors, small cracks and crevices require closer attention. The niche areas could represent potential hiding spots for species and should be examined with care, especially when coming across a vessel that shows high levels of fouling.

Aiming for the future, there has been some discussion of the possibility to restrict or even prohibit the use of copper based antifouling paints in leisure crafts in the Baltic Sea region, due to the harmful effect of Cu in nature (Lagerström et al. 2018). A recent initiative towards such a progression was made by the CHANGE -project, funded by BONUS 2014–2017. The projects policy recommendations include the phasing-out of biocidal antifouling products in the Baltic Sea on leisure crafts before 2030 (BONUS CHANGE Summary report, 2018). Therefore, examining active vessels for non-indigenous species might be more crucial than before.

3.1 Material and methods

3.1.1 Estimation of fouling rate

The fouling rate of the hull should be determined at the end of the boating season in agreement with the boater as the boat is taken up and prepared for winter. The fouling rate should be estimated on each vessel according to a ranking system (Table 2). The observed area should only include the boat surface that has been submerged, below the waterline. If the boat is not taken up, the estimation should be done for the surface area visible underwater. There is currently no ranking

system developed specifically for the Baltic Sea region. The ranking system used in this protocol was introduced in a study by Floerl et al. in 2005 to estimate the fouling rate of boat hulls in New Zealand. This model is intended for vessels that are in the water but was considered just as effective when analysing vessels out of sea. Therefore, only minor modifications in the fouling descriptions were executed on the original ranking system.

When estimating the fouling rate, the length of the vessels should be recorded or at least reported in categories (**0 to 5 m**, **5 to 10 m** and **10 to 24 m)** to gain a rough estimation of the fouling surface area, since it is practically impossible to sample and analyse the entire coverage. Vessels over 24 m are not considered as recreational boats according to the EU Directive 2013/53/EU. The length of the boat could have an effect on the vessel's potential to carry and spread NIS species, since surface area increases with vessel size, thus providing more area for biofouling.

Table 2. Fouling scale ranks based on the ranking system by Floerl et al. (2005).

Rank	Description	Visual estimate of fouling cover
0	No visible fouling. Hull entirely clean, no biofilm on the previously submerged parts of the hull.	Nil
1	Slime fouling only. Previously submerged hull areas partially or entirely covered in biofilm, but absence of any macrofouling.	Nil
2	Light fouling. Hull covered in biofilm and 1–2 very small patches of macrofouling (only few taxa present).	1–5 % of surfaces that have been submerged
3	Considerable fouling. Presence of biofilm, and macrofouling still patchy but clearly visible and comprised of either one single or several different taxa.	6–15 % of surfaces that have been submerged
4	Extensive fouling. Presence of biofilm and abundant fouling assemblages consisting of more than one taxon.	16–40 % of surfaces that have been submerged
5	Very heavy fouling. Diverse assemblages covering most of the hull surfaces.	41–100 % of surfaces that have been submerged

3.1.2 Photographing the fouling areas and documentation of vessel

Simultaneously with the fouling rate estimations, the fouling species on boat hulls should be photographed for later identification after boating season. In this protocol the photographing methodology follows close to that of Zabin et al. 2014. In the respective study all observations were conducted underwater, but the methodology is also suitable to be used on vessels on land. The recommended procedure in this protocol is to photograph the boats when they are lifted from the water and prepared for the winter. In case the boats are not lifted after the season, the photographing should be done with an underwater camera mounted on an angled pole as according to Zabin et al.

The size of one photographed hull surface area should be 8 x 12 cm (Zabin et al. 2014). Depending on the fouling rate estimations (Table 2), there are two ways of photographing the hull surfaces. If the fouling rate is ranked 0-3 (no visible fouling - considerable fouling), photographs should be taken of random fouling patches covering the hull, yet eight photographs in maximum.

If the fouling rate is ranked 4-5 (extensive fouling - very heavy fouling), photographs should be taken along transect lines. The first transect line should be set on one side of the hull running from bow to stern just below the waterline (Figure 4). Eight photographs of the hull surface along the transect line should be taken randomly. The size of one photographed area should be 8 x 12 cm. A parallel transect line should be set at the bottom of the craft as near to the keel line as possible. Another eight photographs should be taken at random sites along this transect.

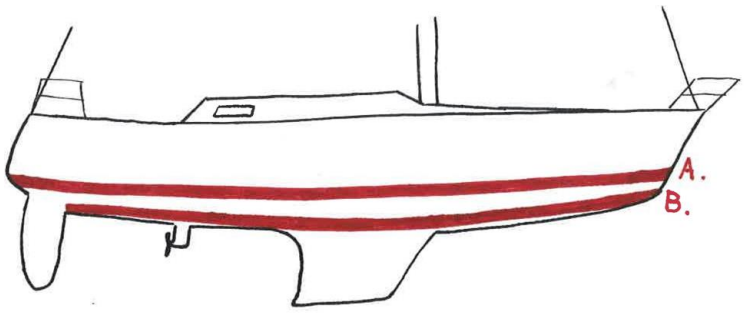

Figure 4. Side view of a boat with two transect lines. The transect line A. is just below the waterline and B. is at the bottom of the boat.

Since many vessels are treated with antifouling paint, the fouling will often only concentrate on specific untreated structures. These niche areas on vessels are: fender, mooring, rudder, ladder, mooring line, water intake, anchors, outboard motors, bow thrusters, anodes and knot meter. The niche areas should be checked for fouling and photographed if biofouling organisms should grow on them. Additionally, small cracks and crevices require closer attention. The photographed area on these niche areas should also be 8 x 12 cm, if possible.

To achieve an overview of the community types growing on the hull, the organisms in the photographs should be identified to the lowest taxonomic level possible and the percentage cover estimated. In case the species identification from photographs is too challenging, the percent cover of coarse taxonomic groups should be estimated.

General information concerning the vessel should be recorded. At least the following information should be recorded**: type of boat, size of boat**, **material and occurrence and type of antifouling paint**. If possible, the boat owner who has agreed to have their vessel's fouling surface photographed should also answer the questionnaire (Appendix 1.) and the answers should be joint with the photographs and samples. Yet it might be a challenge to get in touch with the boat owners, since they might not be present when the boat is lifted and prepared for winter by a respective company.

3.1.3. Sampling of leisure craft

When possible, sampling of leisure craft is highly recommended. Photographing vessels will only give a general idea of the overall fouling rates and very few species can be recognized by photographs alone. Scraping samples are therefore a necessary part of examining the vessels. The biggest issue is that permits may be hard to receive, since not all boat owners are present when their boats are lifted out of the water for the winter.

Scraping samples should be taken *after* the vessel has been photographed. Since fouling can be scarce due to usage of antifouling paint, samples should be taken from several niche areas to give an as accurate indication of the species community as possible. The scraping samples should be taken with a spatula on a small (ca. 2.0 x 2.0 cm) area. Depending on the fouling rate estimations (Table 1), if the fouling rate is ranked 0-3 (no visible fouling - considerable fouling), scraping samples should be taken of random fouling patches covering the hull and the niche areas. If the fouling rate is ranked 4-5 (extensive fouling - very heavy fouling), eight samples should be taken along each transect line that were used when photographing the hull. Along with the hull samples, the niche areas should be sampled, too. Optimally, the samples should be labelled and stored in separate sampling jars. The samples should be preserved in e.g. ethanol. The sampled species should be identified to the lowest taxonomic level possible with a microscope. In case an unknown species is found, it should first be photographed and then preserved for further studies.

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APPENDIX 1.

Biofouling survey and boater questionnaire

Questionnaire Biofouling of leisure boats

The questionnaire is available online in different languages:
https://linmantis60.bsh.de/limesurvey/index.php/835883?lang=en

The link to the questionnaire will also be published on the COMPLETE project home page:
www.balticcomplete.com

The printable English version of the Questionnaire

About

Within the EU INTERREG Baltic Sea Region project COMPLETE we are collecting data on biofouling of leisure boats, antifouling strategies, cleaning procedures and facilities. The aim of the project is to compile information on best practices and deliver knowledge and user-oriented tools for efficient regional biofouling management in the Baltic Sea Region.

For this purpose, cooperation of local marinas and boat owners is essential. We would be grateful for your knowledge and advice. Please, take 10 minutes of your time to fill out the form. Thank you in advance!

Date survey completed _____/_____/_____ (dd/mm/yyyy)

Part A: Boat details

1. Type of boat:

□ Sailboat

□ Powerboat/Motorboat

□ Fishing boat

□ Other (Please, specify!) _____

2. Length of boat:

□ 0 to 5 m

□ 5 to 10 m

□ From 10 to 24 m

□ Over 24 m

3. Hull type:

□ Wood

□ Aluminium

□ Fiberglass

□ Other (Please, specify!) _____

4. Do you use a trailer?

□ Yes

□ No

5. Do you use the same trailer in sea **and** fresh water?

□ Yes

□ No

Part B: Use of the boat and main mooring place (with reference to the last sailing season)

Home port/ main port: _____

1. How often did you use your boat during the last sailing season?

□ Daily

□ Weekly

□ Monthly

□ Occasionally (less than monthly)

□ Never

2. Was your boat moored at any kind of structure (port, marina, yacht club, landing stage etc.)?

□ Yes

□ No

3. If yes, where did you moor your boat?

□ It was moored for the entire season at the same location (port, marina or others).

□ It has a main location, but it was moored at different locations during the season.

□ It does not have a main location; it was moored at different locations during the season.

Please, give the names of mainly used locations: _____

4. How much time did your boat spend at the main location?

□ All year long

□ All sailing season long

□ Part of the sailing season

□ Occasionally

Please, specify in which location: _____

Average lay time (in days): _____

Longest lay time (in days): _____

4. How often did you get your boat out of the water during the sailing season?

□ Daily

□ Weekly

□ Monthly

□ Few times a season, (less than monthly)

□ Only at the end of the season

□ Never

Part C: Antifouling

Definition **antifouling system** (by IMO - International Maritime Organisation): Anti-fouling system means a coating, paint, surface treatment, surface, or device that is used on a ship to control or prevent attachment of unwanted organisms.

1. Is the boat treated with any kind of antifouling system?

□ Yes

Name of applied antifouling -system (in case of paint self-mixture, please indicate the composition!): _____
If applied, please indicate the concentration of biocide:
Date of application: _____

□ No

□ I don't know, I have just bought the boat / the boat is not owned by me.

2. Do you apply a different or special antifouling system of niches, tubes, propeller etc.?

□ Yes (Please, specify!): _____

□ No

3. How often do you apply/renew the antifouling system?

□ Every 6-10 years

□ Every 4-5 years

□ Once every 2-3 years

□ Once a year

4. Do you make use of consultation for necessity, choice and application of antifouling systems?

□ Yes (Please, specify!): _____

□ No, I do it on my own.

5. Are there any regulations for the application of antifouling systems or enforcement implemented in your place?

□ Yes (Please, specify!): _____Which administration is involved? :_____

□ No

□ I don’t know.

Part D: Cleaning

1. How often do you clean your boat of biofouling (cleaning intervals)?

□ Several times a year

□ Every 12 months

□ Every 24 months

□ Every 36 months

□ Every 60 months or less often

2. Where do you clean your boat (cleaning facility)?

Place, country: _____

Type of the facility (if possible, please name the facility): _____

3. Which cleaning technique do you use? (several answers possible)

□ Routine hull cleaning with hard and soft brushes / sponges

□ Professional hull cleaning with pressure washer / disc sander

□ Professional hull cleaning followed by antifouling painting

□ None

□ Other cleaning technique (Please, specify!): _____

4. Where do you carry out the cleaning of your boat?

□ In water

□ On boat ramp

□ In dry dock

□ On land

□ Other (Please, specify!): _____

5. Where is the removed material from the hull disposed?

□ In water

□ Recycling waste containers

□ I do not know (I do not personally clean the boat).

□ Other (Please, specify!): _____

Part E: Recommendations/experiences

Please, provide the following recommendations based on your experience:

1. Antifouling system recommended: _____

2. Cleaning procedure recommended: _____

3. Cleaning facility recommended: _____

4. Other recommendations/best-practice/experiences: _____

5. Please, also provide information on bad experiences with antifouling systems, cleaning etc.:

_____

Part F: Journeys (with reference to the last sailing season)

Please, indicate the type of journey for each area: none, short (daily trips from the home marina), weekender (few day trips), long trips (to one destination/port further away), long tours (long trips with multiple destinations/ports, staying always for only a few days).

	None	Short	Weekender	Long trips	Long tours
Baltic Sea:					
Denmark	○	○	○	○	○
Germany	○	○	○	○	○
Poland	○	○	○	○	○
Russia (Kaliningrad)	○	○	○	○	○
Russia (Petersburg)	○	○	○	○	○
Lithuania	○	○	○	○	○
Latvia	○	○	○	○	○
Estonia	○	○	○	○	○
Finland	○	○	○	○	○
Sweden	○	○	○	○	○
Other:					
North Sea	○	○	○	○	○
Norwegian Sea	○	○	○	○	○

Mediterranean Sea	○	○	○	○	○
Red Sea	○	○	○	○	○
Black Sea	○	○	○	○	○
North Atlantic Ocean	○	○	○	○	○
Extra/Other destinations	○	○	○	○	○

2. How many days was your longest trip? (from start point to destination) .....

3. What was your average velocity? .....

4. How many voyages (leaving the main port) do you have per year? .....

Part G: Non-indigenous species in the Baltic Sea

1. Have you ever heard about the problem of the biological invasions by marine organisms?

□ Yes

□ No

□ Yes, but not in the Baltic Sea

2. Have you ever seen unusual organisms, perhaps non-indigenous species, attached on your boat?

□ Yes

□ No

3. Which part of the boat might be capable to transport and spread organisms in your experience?

□ The hull

□ The anchor and other awash elements of the boat

□ Onboard areas of the boat with stagnant water

□ Other (Please, specify!): _____

4. Have you ever thought of reporting an "unknown" species to any marine authority?

□ Yes

□ Yes, but I do not know to whom to report it.

□ No

5. Are you familiar with the IMO guidelines GUIDANCE FOR MINIMIZING THE TRANSFER OF INVASIVE AQUATIC SPECIES AS BIOFOULING (HULL FOULING) FOR RECREATIONAL CRAFT?

□ Yes

□ Yes, and I apply the guidance (Please, outline shortly your experiences!): _____

□ Yes, but I do not apply any guideline.

□ No

The compilation of the data of this questionnaire is carried out anonymously.

Nevertheless, if you want to get in contact with the COMPLETE project team and for further information please contact:

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THANK YOU VERY MUCH FOR YOUR COOPERATION AND SUPPORT!

APPENDIX 2.

Equipment list for settling plate deployment and field sampling

Settling plates and scraping samples:

- plate sets
- buoys (if needed)
- labels with contact information (attached to plate sets)
- labelled zip lock bags for the plates and
- jars for the scraping samples
- salinometer
- hand dredge
- hand held depth sounder
- ethanol (or other)
- field protocol
- GPS tracker
- extra cord
- cooler with cold blocks for the samples

Post season examination of boat hull fouling levels:

- digital camera
- 25 m transect line (labelled at 1 m intervals)
- frame (8.0 x 12.0 cm)
- spatula
- labelled jars for the samples
- field protocol
- ethanol (or other)

APPENDIX 3.

Contact information

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