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Jonny Beyer & Rolf C Sundt

A brief literature review of *Hediste diversicolor* (Müller, 1776)

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Rolf C Sundt — Sign date 7/11-06
Project Manager

Steinar Sanni — Sign date 8/11-06
Project Quality Assurance

Päivi A Teivainen-Lædre — Sign date 7.11.06
Sr. Vice President
(IRIS – Marine Environment)

Preface

This review report was made by IRIS - Marine Environment (Stavanger, Norway) on assignment from Akvamiljø Caspian.

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Summary

Around 1940, the polychaete ragworm *Hediste diversicolor* (Müller, 1776) (formerly *Nereis diversicolor*) was imported to Caspian Sea from the Azov Sea in the Berdyansk region (Ukraine). Soon the species became established over a large area along the Caspian Sea coast. Then, after 1995, the species was found at offshore oil production locations approximately 30 km off the coast. The questions are: how did the species spread out to the offshore locations, and could this colonisation at the offshore locations possibly represent any environmental concern? The present report includes a brief review of the available knowledge related to the biology of this ragworm species. Our objective is to provide a collection of knowledge which can assist the process of addressing the above noted questions. Based on the reviewed information we also suggest in short how this topic should be approached in terms of practical surveys. I.e., the very first topic which needs to be clarified is whether or not *H. diversicolor* populations are present in the area between the shore locations and the offshore platform locations. Since the species has a limited dispersal capacity a gap in presence would indicate that the dispersal out to the offshore locations has been assisted by human actions, and we would in that case initially consider ballast water in tankers to be the most likely vehicle for species dispersal. Furthermore, a full elucidation of such a dispersal process is possible, and would best be obtained by means of using analyses of population specific molecular markers in representative polychaete samples collected from each of the populations studied (offshore and coastal). Lists of expert and expert laboratories which have competence within these analyses are included in the present report.

1 Introduction

The polychaete ragworm *Hediste diversicolor* (Müller, 1776) (formerly *Nereis diversicolor*) was not native to the Caspian Sea before it was introduced there around 1940. The species was imported from the Azov Sea (Berdyansk region, Ukraine) in an attempt to restructure the benthic ecosystem and to serve as food for sturgeons (Khlebovich and Komendantov 2002). A similar introduction took place in the Aral Sea in 1960. In less than 10 years after the introduction the worm had become established over 30 000 km^2 especially in the NW area of the Caspian Sea. The species is now widely distributed in all Caspian shallow waters. Prior to 1995 the species were not recorded at the offshore locations, but during recent monitoring surveys at offshore oil production platform locations within the ACG and Shah Deniz contract areas the species was found at all offshore study locations.

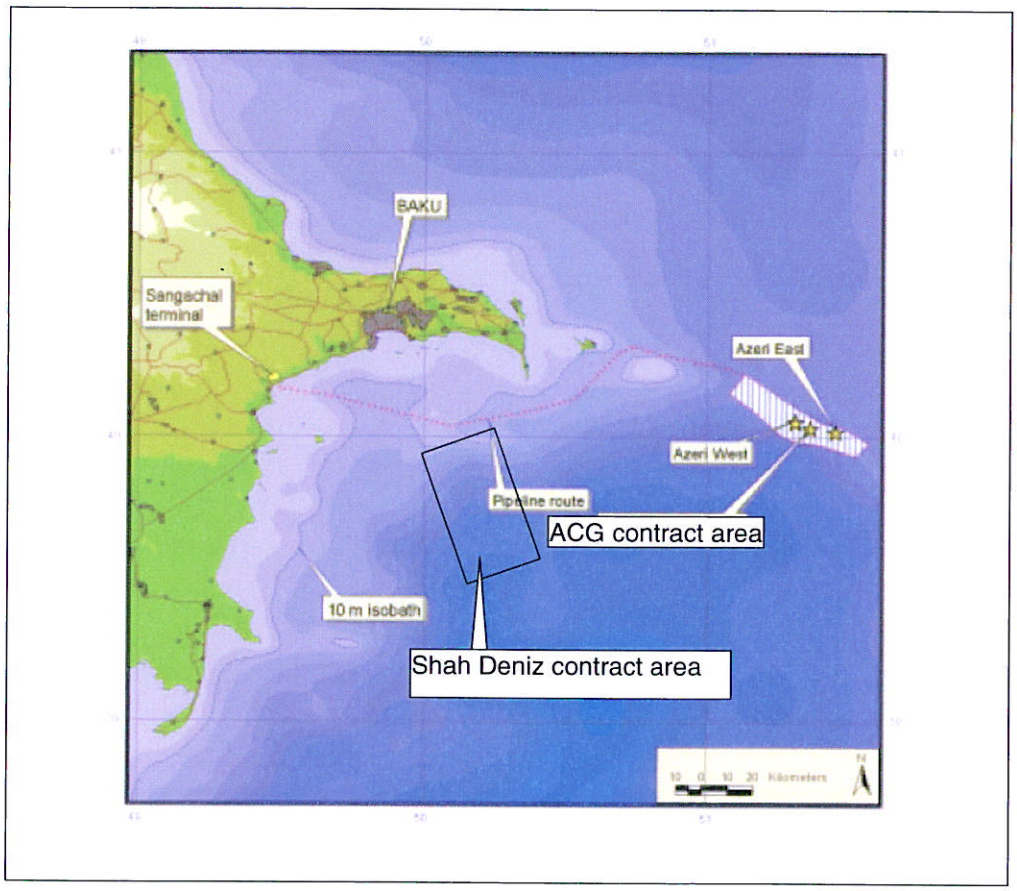

Figure 1: Offshore locations in the Caspian Sea in which the presence of the common ragworm *Hediste diversicolor* is recorded.

In this report, a summary of relevant knowledge about *H. diversicolor* is collected. The objective is to provide a basis for assessing the process whereby this species has

colonized the offshore locations in the Caspian Sea and also to provide a basis for assessing whether this dispersal can have environmental impact on the ecology of the offshore locations in the Caspian Sea.

1.1 Scope/issue of the present report:

1. To prepare a short literature review about the biology of *H. diversicolo*r, with special emphasis on:
   a. Taxonomy and nomenclature
   b. Behaviour and ecological role
   c. Reproduction, life cycle and dispersal of larva
2. To make an assessment of the need for and best available methods for taxonomic DNA analysis of the *H. diversicolo*r species.
3. To make a summary of European expert groups of *H. diversicolo*r biology.

2 The biology of *Hediste diversicolor*

H. diversicolor is a common ragworm which lives in waters of a wide range of salinities (euryhaline). The elongated and flattened body has conspicuous parapodia (Gr. para, beyond or beside + podia, feet) equipped with bristles. As adults it can be up to 12 cm long. The head has four eyes, two antennae, two palps and an eversible pharynx armed with chitinous teeth. The typical colour is greenish during the spawning season and varies from red to light brown at other times. It is common in sandy and muddy sheltered environments in the intertidal region and is widely distributed in euryhaline habitats throughout northwest Europe, in the Baltic Sea, North Sea, and along Atlantic coast to the Mediterranean.

Figure 2: *Hediste diversicolor* (Photo: Anders Ruus, NIVA, with permission)

The biology of *H. diversicolor* is much studied, and the available literature about the species is considerable. A non-selective search in the ISI-database using "*Nereis diversicolor*" as search words yields more than 500 hits referring to refereed papers in scientific journals. In addition, there are numerous books made about the biology of this species and its closest polychaete relatives. The comprehensive review paper about the biology, ecology and potential use of *H. diversicolor* issued in 2002 by P. Scaps included the use of 136 cited references (Scaps 2002).

2.1 Phylogeny and nomenclature

H. diversicolor belongs to Phylum: Annelida, Class: Polychaeta, Order: Phyllodocida, and Family: Nereididae. The *H. diversicolor* species was described first in the Baltic Sea by O.F. Müller (1776) and was originally named *Nereis diversicolor*. Presently, the *Nereis diversicolor* name is still much in use, but apparently the use of the name *Hediste diversicolor* gets more and more common in particular as taxonomy data of this and closely related species using molecular analyses of proteins (Scaps et al. 2001) and genetic materials (Knowlton 2000)gets available.

Nereids are in general well known organisms but the exact identification of species are often difficult. Nereidids were already mentioned in pre-Linnean writing (Fauchald and Rouse 1997). *Nereis pelagica* (Linné, 1758) was among the first polychaete species described, and this species was later designated to be type species in the genus Nereis, which also is the type genus of the Nereididae family. In 1865, Johnston was the first author to diagnose the family and give it a formal description (Johnston 1865). Web-pages with helpful information on anatomy, morphology and taxonomic determination of Nereids is now relatively easy available across the Internet, e.g. (Bakken 2003).

Due to the great morphological homogeneity occurring within the Nereididae this family includes many sibling species; i.e. species that are not morphologically distinguishable but which cannot interbreed (Scaps 2002). The detailed taxonomy and nomenclature of the genus, subgenus, species and subspecies of *H. diversicolor* is a matter of considerable debate among taxonomist. The disagreement is partly due to the great variability of morphological, biochemical and physiological features noticed in individuals from different area-populations and among individuals found in the same area but living at different environmental conditions. Some taxonomists still think the *H. diversicolor* species belongs to the genus *Nereis* with *Hediste* as a subgenus, whereas others think *Hediste* ranks as a genus in its own right, and others again consider *Hediste* to be irrelevant and should not be used at all (Read 1999). To conclude, the old name *Nereis diversicolor* will apparently be used much also in the future, but the appropriate manner to write the species name is *Nereis (Hediste) diversicolor* if the former name is used, and in that case *Hediste* is considered as the subgenus. The other alternative is to use *Hediste diversicolor* if the new nomenclature is used, and in that case *Hediste* is considered to be a separate Genus away from the other *Nereis* species. In the present report, we decided to utilise *Hediste diversicolor* as the name.

For correct nomenclature, the name of the person who first described the species should be placed immediately after a species name, i.e. *Nereis diversicolor* (Müller, 1776). The

name of Müller (1776) as the species-describing author should be used also if any of the more recent name alternatives shown above are used, e.g. *Hediste diversicolor* (Müller, 1776). In a text, the inclusion of the name of the species-describing author is normally done only the first time the name is included in the text (in addition to in the title and in the abstract if the name is used there). In cases referring to other authors together with the species-describing author there must be a clear split between the authors, such as *Hediste diversicolor* (Müller, 1776), (see Read 1999). After spelling out the full name the first time the species is named an abbreviation of the genus name is often used in the rest of the text, e.g. *H. diversicolor*.

2.2 Basic anatomy and taxonomy

The generic characters used for morphological identification of Nereids are: prostomium (shape), antenna (number, shape), tentacular cirri (number), general shape of main body, parapodia (shape, differs often along the body), setae at the parapodia (type, form, differs along the body, setal signature), paragnath and papilla at the eversible pharynx (presence, number, form, arrangement pattern).

According to (Glasby 1993) and (Bakken 2003), the detailed anatomical/morphological characteristics of Nereidids are as follows: Lateral antennae, 1 pair (rarely absent); median antennae absent (rarely present); palps eversible; nuchal organs not externally exposed. Peristomium fused with first segment; 2 pairs of peristomial cirri (anterodorsal and anteroventral); segment 1 cephalised and carrying 1-2 pairs tentacular cirri without aciculae (distinction between peristomial and tentacular cirri apparent during development). The pharynx partially differentiated. A pair of lateral jaws is present and usually accessory denticles or papillae. Notopodia distinct (rarely reduced), usually with more flattened lobes, notosetae compound falcigers and/or spinigers (rarely notosetae absent); neurosetae compound falcigers and often compound spinigers, shafts internally with mediculla and cortex, present in subacicular patch and supra-acicular fascicle; anal cirri paired (Glasby 1993). According to Glasby, the Nereididae is a natural monophylic group. Evidence for this monophyly is the autapomorphic characters distinct notopodia, and notosetae with compound falcigers and/or spinigers. There are currently acknowledged three subfamilies of the Family Nereididae, including the Nereidinae which is characterised by a pharynx with chitinous paragnaths (small, horny, tooth-like jaws which are typical for certain annelids), and papillae sometimes present (Hutchings and Reid 1990).

H. diversicolor is determined morphologically based on: (A) An eversible pharynx with conical paragnaths on both rings. (B) Four pairs of tentacular cirri and biramous (double-branched) parapodia. (C) Notosetae homogomph spinigers. Neurosetae homo- and heterogomph spinigers; heterogomph falcigers. (D) A single homogomph falciger present in median and posterior neuropodia (Fauchald 1977; Fauchald and Rouse 1997; Bakken 2003). However, several species in the Hediste subgenus (e.g. *H. limnicola and H. japonica*) are morphologically similar to *H. diversicolor* and these are believed to constitute a species complex with *H. diversicolor* (Fong and Garthwaite 1994). The issue of eventually clarifying all taxonomical and morphological details within the

Nereis genus and Hediste subgenus remains to be done by taxonomy experts, and this issue will not be further discussed in this report.

Other papers and books which contain taxonomical information of *H. diversicolor*: (Chambers and Garwood 1992; Dahlgren et al. 2000; Khlebovich and Komendantov 2002; Bakken and Wilson 2005; Hesselberg and Vincent 2006).

2.3 Behaviour and ecological role

H. diversicolor is very common species in the shallow marine and brackish waters in the North Temperate Zone of the Atlantic. It displays a solitary and infaunal behaviour by building conspicuous U or Y-shaped burrows in the soft sediments (Davey 1994; Scaps 2002). Although being an infaunal species it is able to swim freely in the water by means of undulating movements, similar as its larger relative *Nereis virens*.

In some areas the density of a *H. diversicolor* population may reach several tens of thousands per square meter (when juvenile worms are considered), but normally an adult population density in the range 100-1000 individuals is common (Scaps 2002). The density and the biomass of the population of decreased during winter and increased during spring/summer (Gillet and Torresani 2003). The burrows serve as sites for foraging and as refuges against predators. The burrows have a significant effect on the surrounding sediment environment since it to a great extent increases the sediment-water interface (Davey 1994; Scaps 2002). When the worms ventilate their burrows, they bring oxygenated water vertically and deep into the soft sediment and this strongly stimulates the total oxygen consumption of the sediment (Wenzhöfer and Glud 2004). A higher microbial and meiofaunal growth occurs in the sediment material alongside the burrows. The burrow behaviour of a worm population has also a significant effect on the fate and bioavailability of pollutant chemicals in the sediment (Petersen et al. 1998; Gunnarsson et al. 1999).

The presence and behaviour of *H. diversicolor* populations have influence on landscape development processes, such as in enhancing the erosion and loss of saltmarsh areas (Paramor and Hughes 2004). Such effects are thought to be related both to the physical effect of the burrows and to the feeding behaviour of the species. The feeding ecology and diet of *H. diversicolor* vary greatly and includes a variety of feeding modes; such as passive suspension feeding; surface and subsurface deposit feeding; active and passive omnivorism; and scavenger preying on small invertebrates, plant materials, or ingestion of sand and mud particles to utilize the attached detritus. It utilizes an eversible pharynx to capture prey. Laboratory results have showed that *H. diversicolor* feed on benthic diatoms species, often in competition with Corophium volutator (Smith et al. 1996).

H. diversicolor and other polychaete species serve an important ecological role as prey organisms for many species of fish, and also birds and crustaceans. Being a desirable food item for fish also make the species very popular as bait for angular fishing. Indeed, large amounts of this species (as well as other Nereidid ragworms) are sold commercially as live bait for fishing or as live food item for use in fish aquaculture. The aim of increasing the food availability for local fish resources was also a main reason why *H. diversicolor* was imported to the Caspian Sea back in 1940.

The presence of *H. diversicolor,* and other sediment burrowing polychaetes, may have a significant impact on the fate of organic and inorganic chemical contaminants in polluted sediments. Studies in PAH polluted sediments have shown that both *H. diversicolor* and *Arenicola marina* enhance the fluxes of sediment-associated PAH and metabolites into overlying water, but to different extents (Christensen et al. 2002). The major pathway of PAH removal from sediments with *H. diversicolor* was release of water-soluble metabolites, probably formed by the metabolism in the ragworm (ibid.). For PCBs, which are more persistent than PAHs towards metabolic degradation, the presence of *H. diversicolor* has been found to enhance the pollutant release with nearly 300 % in comparison to sediments without containing the worm (Gunnarsson et al. 1999). Also for the fate of heavy metals in contaminated sediments, the bioturbation activity by *H. diversicolor* and other infaunal species have significant influence, for reviews see (Bryan and Langston 1992; Reish and Gerlinger 1997).

2.4 Life cycle, reproduction and dispersal of larva

Life history characteristics (longevity, spawning season, feeding tactics and growth) and population dynamics (sex ratio, density and biomass) vary greatly according to geographical location of the populations (Abrantes et al. 1999; Garcia-Arberas and Rallo 2002; Scaps 2002).

H. diversicolor is gonochoristic (with sexes separate), oviparous (producing offspring in eggs), and reproduces only once in entire life. Adults reach sexual maturity in one to three years. Maturation and spawning are influenced by temperature and lunar periodicity in spring, and during this phase, males are brighter green and females are darker green. However, the *H. diversicolor* remain atokous during the reproduction period, i.e. it does not, unlike many other Nereidid species, change into epitokous forms which are very active, specialised for better swimming and mate searching and which are completely filled with gametes for spawning. The spawning time varies greatly with region within the spring/summer/early autumn period, and considerable variation in spawning time even over short distances is recorded (Abrantes et al. 1999; Scaps 2002).

During the spawning activities, reproductive pheromones coordinate essential processes such as mate location and synchronism for release of gametes when males discharge sperm around burrow of females. The female then fertilises the eggs inside the burrow by ventilating the sperm containing water over them. The eggs are large > 0.2 mm and after fertilisation they stay under the female for brooding. The larva is lecithotrophic (lives off yolk supplied via the egg), and during the incubation, the larvae and post larvae remain under the body of the female for at least 10 days (Marty and Retiere 1999; Scaps 2002). The hatching occurs at the trochophore stage and the larvae normally remain in the female burrow until they reaches a size of 7 or 8 segments. At this stage the female dies. After emergence from the parental burrow, the juveniles adopt a morphology and behaviour very similar to the adults (Marty and Retiere 1999). The degree of which the larva of *H. diversicolor* is spread is therefore limited in comparison to polychaete species that have a longer mobile and more pelagic stage of their larvae. For example, *Nereis virens* has a true pelagic larval phase that may last as long as 9 days or more (Breton et al. 2003).

3 Molecular methods for taxonomic determination

Taxonomic keys for identification of *H. diversicolor* and other related species in the Nereididae family have been based on classical methods using anatomical and morphological characteristics as summarised earlier in the text. However, the general homogeneity of many of these traits within the Nereididae family combined with a large degree of variability in morphological traits (phenotypic plasticity) within species has hampered the precision of this approach. More recently, new identification methods using bio-molecular and biochemical markers, including polymorphism analyses of specific proteins and enzymes and DNA sequence information and molecular characteristics of mitochondrial DNA, have been developed. These molecular methods can be used both for discriminating between sibling species, but also for studies of small differences between different populations and for studies of temporal changes within one population.

3.1 Polymorphism of tissue proteins and allozymes

The separation of polymorphic proteins by electrophoresis will often yield very specific protein patterns which can be used for species identification. A much used method is IsoElectric Focusing (IEF), which is an electrophoretic separation of proteins based on their isoelectric points (pI). The pI is the pH point at which the protein has an overall net charge of zero, and differences of only a few hundredths of a pH-unit in isoelectric points, e.g. due to slight variations in amino acid sequence, are sufficient to resolve proteins or polymorphic protein forms from each other. IEF can also be used in combination with SDS-PAGE electrophoresis, which is based on size of proteins, as it is done in 2D-electrophoresis.

Allozyme polymorphism is another molecular approach much used for species identification and population studies in annelids. Allozymes are defined as polymorphic forms of an enzyme that differs in amino acid sequence, as shown by electrophoretic mobility or some other property, from other forms of the same enzyme and is encoded by one allele at a single locus. Virgilio & Abbiati studied the temporal genetic changes in six allozyme loci in intertidal populations of *H. diversicolor*; namely ALD, (aldolase), FH (fumarate hydratase), HBDH (hydroxybutyrate dehydrogenase), LDH (lactate dehydrogenase), PGI (phosphoglucose isomerase), SDR (short-chain dehydrogenase/reductase). The parameters studied; allelic frequencies, percentages of polymorphic loci, mean observed and expected heterozygosities, were found to vary both between sampling times and among sites. As indicated by allozyme studies, the population densities of *H. diversicolor* are apparently primarily affected by periodical mortality events followed by recruitment peaks. Genetic drift, related to mortality events, and/or sweepstake reproductive success, with a small number of individuals responsible for the recruitment within patches, appear to be major processes promoting the genetic structuring of *H. diversicolor* within estuaries (Virgilio and Abbiati 2006).

Other studies of *H. diversicolor* using allozyme determinations: (Hateley et al. 1992; Fong and Garthwaite 1994; Abbiati and Maltagliati 1996; Rohner et al. 1997; Virgilio et al. 2003; Virgilio and Abbiati 2004; Virgilio et al. 2005).

Collins *et al* determined the amino acid sequence of soluble, sarcoplasmic, Ca2+-binding proteins (SCBPs) in *H. diversicolor* muscle tissue (Collins et al. 1988). This protein belongs to the calmodulin superfamily and contains four homologous domains (I-IV). It is common in all invertebrates. Collins *et al* found that SCBP in *H. diversicolor* is a single polypeptide chain of 174 amino acids, including single residues of glutamine and histidine, 2 tyrosines, and 3 tryptophans. It is devoid of cysteine and has an acetylated amino terminus, a calculated Mr of 19,485, and a net charge of -13 at neutral pH. They found no evidence for heterogeneity in the sequence and probable Ca2+-binding sites were recognized in domains I, III, and IV. Comparison with other available invertebrate SCBP sequences indicated a high degree of variability among these proteins between species, with only 9 residues common to all species (Collins et al. 1988). The analysis which yields species-specific protein patterns that depend on the characteristic SCBP amino acid sequance and is mainly performed by electrophoresis; in most cases isoelectric focusing (IEF) (Rehbein 1990). The method is robust. Protein dry powder samples, which are prepared from the sarcoplasmic fraction, which are stable at room temperature and can be shipped without cooling, can be used.

Other studies of *H. diversicolor* using determination of sarcoplasmic calcium-binding proteins (Babu et al. 1987; Cook et al. 1991; Vijaykumar and Cook 1992; Dekeyzer et al. 1994; Craescu et al. 1998; Sillen et al. 2003).

3.2 Species identification using DNA and gene analyses

Analyses of DNA sequences and other genome level traits can be used as powerful tools for phylogenetic studies, for species identification and for discriminating sibling species. Since estuarine environments tend to restrict gene flow and impose distinct selective regimes, in particular for species with limited dispersal capacity, physiologically adapted populations which are genetically divergent from each other and from their marine counterparts are produced (Bilton et al. 2002). Several genes and gene-families are used for molecular systematic studies and DNA sequencing in polychaetes over the past couple of years, including the 18S rRNA gene and the EST gene family. The ETS family includes a growing number of transcription factors with a highly conserved DNA-binding domain, the ETS domain. Already in 1994, Lelievrechotteau et al used PCR amplification with degenerated oligonucleotides to isolate two putative ETS DNA-binding coding domains in H. diversicolor (Lelievrechotteau et al. 1994). Breton et al studied population genetic structure of *H. diversicolor* by using DNA sequence data of the mitochondrial gene cytochrome b (cyt b) (Breton et al. 2003). The results showed that widely separated *H. diversicolor* populations were strongly differentiated at the cyt b locus (Breton et al. 2003).

Other studies of *H. diversicolor* using DNA sequence and gene analyses: (Dekeyzer et al. 1994; Lelievrechotteau et al. 1994; Depledge 1996; LelievreChotteau et al. 1996;

Bocquet-Muchembled et al. 1999; Knowlton 2000; Bocquet-Muchembled et al. 2001; Bocquet-Muchembled et al. 2002).

4 European experts and expert groups of *H. diversicolor* biology

Table 1: Scientific experts and expert laboratories on relevant subjects regarding the common ragworm *Hediste diversicolor.*

Competence	**Expert person / group & Address**	**Refs.**
General biology, phylogeny, ecology and larval development of *Hediste diversicolor*	Scaps, P, Univ Sci & Technol Lille, Lab Ecosyst Littoraux & Cotiers, CNRS, Upres A 8013, F-59655 Villeneuve Dascq, France.	(Scaps et al. 1996; Scaps et al. 1997; Scaps and Borot 2000; Scaps et al. 2001; Scaps 2002; Rouabah and Scaps 2003)
	Moreira SM, Univ Aveiro, Dept Biol, P-3810 Aveiro, Portugal.	(Abrantes et al. 1999; Moreira et al. 2005; Moreira et al. 2006)
	Marty, R, Museum Natl Hist Nat, Stn Marine Dinard, 17 Ave George V BP 70134, F-35801 Dinard, France.	(Marty and Retiere 1999)
	Torkild Bakken, Norwegian Univ Sci & Technol, Sect Nat Hist, NO-7491 Trondheim, Norway.	(Bakken and Wilson 2005)
Molecular markers for species determination and population genetic studies of *Hediste diversicolor*	Virgilio, M, Univ Bologna, Ctr Interdipartimentale Ric Sci Ambientali, Via S Alberto 163, I-48100 Ravenna, Italy. mvirgilio@ambra.unibo.it	(Virgilio et al. 2003; Virgilio and Abbiati 2004; Virgilio and Abbiati 2004; Virgilio et al. 2005; Virgilio and Abbiati 2006)
	Knowlton N., Univ Calif San Diego, Scripps Inst Oceanog, Marine Biol Res Div 0202, La Jolla, CA 92093 USA.	(Knowlton 2000)
	Breton S, Univ Quebec, Lab Biol Evolut, Dept Biol, 300 Allee Ursulines, Rimouski, PQ G5L 3A1 Canada	(Breton et al. 2003)
	Dahlgren, T.G., Univ Gothenburg, Dept Zool, S-40530 Gothenburg, Sweden.	(Dahlgren et al. 2000)

5 Summary discussion

The present review report provides information about the biology of *H. diversicolor*, to serve as a knowledge basis for investigations into possible dispersal of this species from the coastal region to the offshore platform locations in the Caspian Sea. In this discussion the most essential elements of the possible dispersal are commented and the most obvious hypotheses and work tools for further studies are suggested.

It is evident from the information reviewed in the present report that this polychaete species generally has a limited dispersal capacity in comparison to most other Nereidid polychaetes. This feature is due to the behaviour of the larva, which stays in the female burrow during most of the time during the development phase. Very soon after the larvae/juvenile worm leaves the female burrow it adopts a burrowing behaviour similar to the older worms. The residence time of the young worm in the pelagic waters is therefore short. The larvae of other Nereidid polychaetes, such as *Nereis virens*, has a much longer resident time (typically more than a week) in the pelagic phase and they will consequently have a better dispersal capacity.

The very first issue which needs clarification in possible further studies is whether or not the species is present in the region between the coast and the offshore production platforms. If the observed dispersal of the *H. diversicolor* species has occurred by natural causes, its limited dispersal capacity will involve that individuals of the species are present also in the area between the coast and the platform locations. This is a distance of approximately 30 km. If the species is found in this area, it would be a clear indication that the dispersal is not attributed to human action but rather due to the normal spread of the species. No further investigations would in that case be needed. Availability of recent sediment samples taken in this area e.g. in connection with pipeline surveys could potentially serve as appropriate samples for such studies.

If the species *H. diversicolor* is NOT found in sediment samples collected recently in the area between the coast and the platforms, it would be more likely that the dispersal process has been assisted by some kind of human action, such as by transport of larva in ballast water of tanker vessels or on hull surfaces. If such assisted dispersal is indeed the case, it is most likely that the dispersal has occurred in form of pre-juvenile larvae, whereas it is less likely that adult individuals have been transported in this way.

This hypothesis, that the dispersal of worms has occurred by accidental transport in ballast water or on ship hulls of operator vessels, can be tested by using the molecular marker methods reviewed above. Also practical information related to the tanker traffic, including which harbours that are used for oil unloading and ballast water loading, are required for the discussion/investigation of such a dispersal process. If tankers operating between the shore and the platform locations unload oil and load their ballast water in one single harbour, the polychaete population inhabiting this harbour location would be the most likely source for the offshore populations. Then, a comparison of specific molecular traits in the selected populations (harbour(s) and offshore location) would possibly clarify their genetic relationship. Several of the expert scientists summarised in Table 1 are the appropriate persons to consult for choosing the appropriate molecular analysis approach and also for conducting the necessary analytical work. It is possible,

however, that an offshore population established due to assisted dispersal has more than one (maybe multiple) source populations. If this indeed is the case, a study using molecular marker methods could fail to produce conclusive results.

5.1 Suggestions of approach for follow up studies

The very first topic which needs to be clarified is whether or not *H. diversicolor* populations are present in the area between the shore locations and the offshore platform locations. Findings of the species all the way through this area would be a stop-signal for further investigations. A "not-present" situation would on the other hand clearly support an assumption that the dispersal of the species has been assisted by the ongoing oil E&P activity in the area. In that case, the next step would be to investigate the most likely mechanism of dispersal, and we suggest that ballast water would possibly be the most likely vehicle for such dispersal of the species, then preferably in form of pre-juvenile larvae. Furthermore, the elucidation of a dispersal process and information about the actual origin of the offshore populations may be obtained in follow-up studies by means of using analyses of molecular (preferably genetic) markers in representative polychaete samples collected from each of the populations studied (offshore and coastal).

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