Minimum Detectable Differences as a criterion to assess the reliability of micro- and mesocosm studies

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Introduction

The recent Aquatic Guidance Document (AGD) for plant protection products (EFSA 2013) requires the reporting of Minimum Detectable Differences (MDD) for micro- and mesocosm studies. The MDD is the minimum difference to the control which is statistically significant. It is further suggested that for at least eight populations of the sensitive group a statistical analysis should be possible to make an isolated study reliable for effect assessment. However, the MDD for a species can differ from sampling date to sampling date and can also depend on the test concentration. Since no specific criteria are given in the AGD on how to evaluate the MDD, Brock et al. (2015) proposed the following criterion:

To allow a reliable evaluation of effects, the MDD should be at least 5 times < 100%, 4 times < 90%, 3 times < 70% OR 2 times < 50 %.

The percentage values were taken from the classification in the AGD for small, medium, medium – large, and large effects.

We present first experience with the MDD concept in the re-evaluation of older mesocosm studies and the use of MDDs in studies recently conducted.

Methods

If not otherwise noted, the MDDs were calculated for the NOEC per taxon and sampling date by the multiple t-test of Williams (α = 0.05, one-sided). Note that the Williams test assumes a monotonous concentration response which is considered appropriate here because the MDD concept is related to direct effects on sensitive taxa. We express the MDD on abundance scale despite that the test itself is based on ln-transformed data to allow the assumption of normal distribution and homogenous variances.

If no additional information was available from laboratory tests we considered algae and macrophytes as potentially sensitive for herbicides, arthropoda for insecticides and all taxa for fungicides and other chemicals.

General information				Statistical design					MDD category 1 taxa (genus or lowest available level)					MDD evaluation						
Nr.	Test system	year	Stressor	total n	Controls	Treatment levels	Replicates	Duration after 1st appl	Algae	Macrophytes	Crustacea	Insects	other Inv	Algae	Macrophytes	Crustaceans	Insects	Other inv	Sensitive MDD Cat 1 pop.	
1	5 m² outdoor mesocosms	2012	F	18	4	5	3 (2)	19	*Chlamydomonas, Chroomonas Microcystis Nitzschia, Volvox*		*Daphnia, Chydorus, Cyclopoidae, Asellus , Crangonyx*	*Chaoborus, Cloeon,* Orthocladiinae, Tanypodinae, Zygoptera	*Keratella (2), Anuraeopsis, Helobdella, Erpobdella,* Oligochaeta, *Physa, Lymnaea , Musculium*	5		5	5	9	**24**	✓
2	5 m² outdoor mesocosms	2013	F	18	4	5	3 (2)	16	Chlorophyceae, Diatomaeae		*Daphnia,* Cyclopoidae, *Asellus , Crangonyx*	*Chaoborus, Cloeon, Chironomus, Polypedilum, Micropsectra, Paratanytarsus, Tanytarsus , Cricotopus , Procladius, Psectrotanypus,* Zygoptera	*Keratella (2),* Oligochaeta, *Helobdella, Erpobdella, Lymnaea, Radix, Physa, Musculium*	2		4	11	9	**26**	✓
3	1.8 m³ outdoor enclosures	2014	F	15	5	5	2	14	*Chlorella, Chromulina, Chroomonas,* Chrysoph. (1-5 µ), Chrysoph (6-10 µ), *Cryptomonas, Ochromonas, Trachelomonas*		*Daphnia,* Cyclopidae, *Asellus*	*Chaoborus, Coenagrionidae, Cloeon, Tanypodinae, Orthocladiinae*	Acari, *Polyarthra, Synchaeta, Keratella,* Lymnaeidae, *Dugesia, Lumbriculus, Helopdella*	8		3	5	8	**24**	✓
4	1.8 m³ outdoor enclosures	2014	F	15	5	5	2	12	*Ankyra, Trachelomonas, Chromulina,* Chrysophyceae (1-5 µ), *Chroomonas, Cryptomonas*		*Chydorus, Daphnia,* Cyclopidae, Diaptomidae, *Asellus*	*Chaoborus,* Coenagrionidae, *Cloeon,* Tanypodinae	*Keratella, Hexarthra, Planorbis, Dugesia*	6		5	4	4	**19**	✓
5	1.8 m³ outdoor enclosures	2006	H	15	5	5	2	12	*Ankyra , Characium , Chlamydomonas (2), Chlorella , Phacomyxa , Tetrachlorella, Rhodomonas, Cryptomonas,* Chrysophyceae (15 µm), Chrysophyceae (610 µm), *Trachelomonas*	*Ceratophyllum , Chara , Potamogeton (2), Stratiotes, Lemna*				12	5				**17**	✓
6	1.8 m³ outdoor enclosures	2009	H	15	5	5	2	12	*Chlorella, Chroomonas ., Chrysophyceae (15µm), Cryptomonas , Erkenia, Nephrochlamys, Nitzschia*	*Ceratophyllum , Chara , Potamogeton, Lemna*				7	4				**11**	✓
7	5 m³ outdoor mesocosms	2010	H	13	3	5	2	8	*Oedogonium, Nitzschia, Chroomonas, Characium*	*Myriophyllum, Potamogeton, Chara, Ceratophyllum, Sparganium, Lemna*	Copepoda (Nauplia), *Daphnia (2)*	*Chaoborus*	*Brachionus*	4	6	2	3	1	**10**	✓
8	1.8 m³ outdoor enclosures	2012	H	16	4	6	2	8		*Ceratophyllum, Chara , Glyceria , Mentha , Myriophyllum , Vallisneria, Lemna , Salvinia , Spirodela, Stratiotes*					10				**10**	✓

Conclusions and Outlook

For most major groups is seems possible to achieve sufficiently low MDDs for at least eight populations in a mesocosm study.

Some taxa can be introduced (e.g. macroinvertebrates, macrophytes). Other taxa are not manageable and especially the structure of the plankton community can be highly variable between studies but also over time within a study. Often only a few species can dominate the plankton community. In-situ bioassays can provide information on survival of additional macroinvertebrate species.

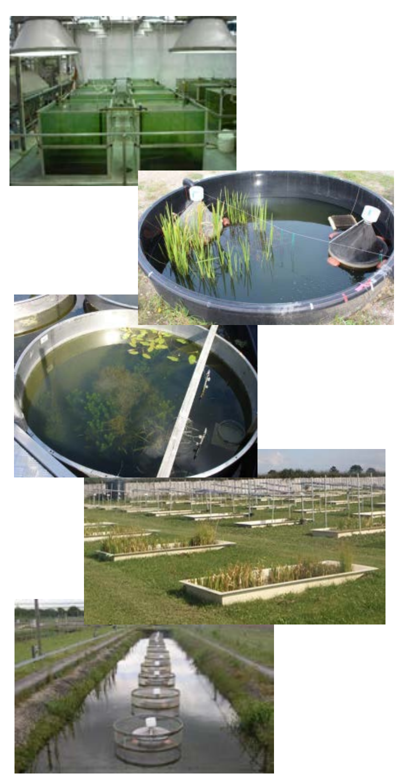
In all cases the MDDs can be reduced by pre-treatment management of the test systems and increasing the sampling efficiency (more sample volume, samplers or traps).

The number of test units or replicates seems to have only a small effect compared to other factors on the number of MDD category 1 taxa.

Results

In total, MDD were evaluated in 20 studies covering tests systems from 1 m³ in the greenhouse over enclosures in larger ponds or ditches up to mesocosms of different design. With respect to the major groups the following results were found.

- **Algae**: Usually only the phytoplankton is taxonomically analysed, periphyton, if at all, in most cases is assessed via chlorophyll a measurements which can allow a differentiation of 3 – 4 major groups. Up to 12 algae populations met the MDD criterion but the final number is given by chance as also less than 8 were found in the same test system using the same methods but in another year.
- **Macrophytes**: Macrophytes growing free in the sediment of the test systems are usually assessed by mapping area coverage. Despite this is only a rough estimation of the biomass, it often provides relatively low MDDs. If a study is focussed on macrophytes potted plants and floating plants in enclosures usually also provide low MDDs. So, achieving 8 species with sufficiently low MDDs seems to be possible.
- **Crustaceans**: Cladocerans and copepods are usually present with a few taxa of sufficiently high abundance in the zooplankton samples. However, copepods are often only determined to the order or family level for practical reasons. From the macrocrustaceans, *Asellus* and *Crangonyx* are often evaluable. These taxa can also be introduced into the systems to achieve sufficient abundances and the sampling efficiency can be optimized by the number and type of samplers used. Most of the test systems used in the past are lentic and thus often not favourable for *Gammarus.* However, *Crangonyx* seems to be a good surrogate of the same order.
- **Insects**: Midges of the family of Chaoboridae and Chironomidae as well as the mayfly *Cloeon* sp. seem to be the default insects allowing an analysis of effects. Chironomids can be differentiated at least into subfamilies depending on their developmental stages**.** In some studies also damselflies could be evaluated and in specific cases, Notonectidae were especially monitored. As for macrocrustaceans, habitat and sampling devices can be optimized to assess insects. However, some orders are usually not present in sufficient numbers to allow an assessment of effects, e.g. Trichoptera, Plecoptera, Coleoptera, Megaloptera. Partly, these taxa might be assessed in lotic systems or in additional bioassays.
- **Other invertebrates:** Only when testing fungicides or other chemicals without a clearly defined target group other invertebrates are considered potentially sensitive by default. In other cases, groups like snails and worms are not considered as potentially sensitive. By the typical macroinvertebrate sampling often at least one snail species, a leech and – if the sediment is sampled - oligochaetes, can fulfil the MDD criterion. In the zooplankton data set mostly one or a few rotifer species can be assessed.



General information				Statistical design					MDD category 1 taxa (genus or lowest available level)					MDD evaluation						
Nr.	Test system	year	Stressor	total n	Controls	Treatment levels	Replicates	Duration after 1st appl	Algae	Macrophytes	Crustacea	Insects	other Inv	Algae	Macrophytes	Crustaceans	Insects	Other inv	Sensitive MDD Cat 1 pop.	
9	6 m³ mesocosms	1999	I	12	3	5	2 (1)	91			*Chydorus , Daphnia ,* Calanoida, Cyclopoida , *Gammarus*	*Chironomus, Micropsectra, Bryophaenocladius , Procladius, Chaoborus, Cloeon*	*Stylaria, Helopdella (2), Asplanchna, Keratella, Polyarthra, Synchaeta*			5	6	7	**11**	✓
10	5 m³ outdoor mesocosms	2004	I	13	3	5	2	13	*Chlorophyceae, Chroomonas, Cryptomonas 1, Cryptomonas 2, Conjugatophyceae , Nitzschia*		*Asellus, Daphnia, Simocephalus, Chydorus,* Cyclopoida, Ostracoda	*Chaoborus,* Chironominae, *Psectrocladius, Holotanypus, Psectrotanypus*	*Stylaria, Gyraulus, Keratella, Polyarthra, Lepadella, Lecane, Synchaeta*	6		6	5	7	**11**	✓
11	5 m³ outdoor mesocosms	2008	I	13	3	5	2	11	*Chromulina, Characium, Chlamydomonas, Chroomonas, Cryptomonas, Gomphonema, Pennales*		*Daphnia, Simocephalus, Chydorus,* Cyclopoida	*Chaoborus, Psectrocladius, Cricotopus,* Chironominae, Tanypodinae	Tubificidae, *Helobdella, Brachionus, Keratella, Polyarthra, Mytilina*	7		4	5	6	**9**	✓
16	1.8 m³ outdoor enclosures		I	15	5	5	2	10			*Asellus, Chydorus, Daphnia, Diaptomidae, Cyclopidae, Gammarus (B)*	*Chaoborus, Cloeon, Chironominae, Tanypodinae, Zygoptera*	*Lymnaea, Planorbidae, Keratella, Polyarthra*			6	5	4	**11**	✓
12	1.8 m³ outdoor enclosures	2014	I	15	5	5	2	10			*Asellus, Chydorus, Daphnia,* Diaptomidae, Cyclopidae, *Gammarus (B)*	*Chaoborus, Cloeon, Chironominae, Tanypodinae, Orthocladiinae, Ceratopogonmidae, Heteroptera, Zygoptera*	*Lymnaea, Planorbidae, Tricladida, Keratella, Polyarthra, Trichotria*			6	8	6	**14**	✓
13	1.8 m³ outdoor enclosures	2014	I	22	6	4	4	12		*Ceratophyllum, Chara , Myriophyllum, Lemna*	*Asellus, Gammarus (B), Daphnia, Chydorus, Calanoidae, Cyclopidae*	*Cloeon, Chaoborus, Phryganea,* Orthocladiinae, Tanypodinae, Anisoptera, Zygoptera	*Keratella, Polyarthra, Dugesia, Lumbriculus,* Naididae, Lymnaeidae		4	6	7	6	**13**	✓
15	5 m³ outdoor mesocosms	2015	I	18	6	4	3	10			*Asellus, Crangonyx,* Cyclopoida, Calanoida, *Daphnia, Simocephalus*	*Chaoborus, Cloeon, Zygoptera,* Culicidae, *Chironomus, Micropsectra, Tanytarsus,* Orthocladiinae, Tanypodinae, Ceratopogonidae	Oligochaeta, *Helobdella, Erpobdella, Anuraeopsis, Keratella 1, Keratella2, Polyarthra, Lepadella, Radix, Lymnea, Physa*			6	10	11	**16**	✓
16	1.8 m³ outdoor enclosures	2007	I	18	4	5	3 (2)	12			*Argulus, Eudiaptomus, Cyclopoidae, Daphnia, Simocephalus, Asellus*	*Chaoborus,* Chironomidae, Baetidae, Coleoptera	*Keratella, Polyarthra, Synchaeta, Lymnaea*			6	4	4	**10**	✓
17	1 m² microcosms in greenhouse	2002/ 2003	I	15	3	6	2	8			*Daphnia (2), Chydorus,* Cyclopoida, Ostracoda		*Keratella (2), Cephalodella, Lepadella*			5		4	**5**	
18	3 m² outdoor mesocosms	2014	Bio-cide	13	3	5	2	10			*Daphnia (2),* Cyclopoida, *Asellus, Crangonyx*	*Chaoborus, Cloeon*	*Harringia, Keratella, Hexarthra, Lymnaea, Radix, Physa, Ologochaeta*			5	2	7	**14**	✓
19	1 m² microcosms in greenhouse	2001	Cu	16	4	6	2		*small Pennales, Rhodomonas, Ankyra*	*Egeria*	*Daphnia (2), Chydorus, Copepoda (Nauplia)*		*Keratella*	3	1	4		1	**9**	✓
20	1 m² cosms in greenhouse	2009 / 2010	Ni	14	4	5	2	16	*Ankyra, Chlorella, Chromulina, Chroomonas, Chrysophyceae (15µm), Coelastrum, Lyngbia, Monosiga, Nephrochlamys*		*Daphnia (2) Acroperus, Simocephalus*		*Lymnaea, Keratella (2), Brachionus, Hexarthra, Chephalodella*	9		4		6	**19**	✓

It should be noted that the definition of the sensitive group comprises arthropods for insecticides, primary producers for herbicides and almost all groups for fungicides (although here soft bodied taxa like molluscs and oligochaetes are usually sensitive). It is true that within a potentially sensitive group some taxa might be more sensitive than others e.g., green algae, Cladocera, or EPT taxa. But these should be considered as sensitive taxa within the potentially sensitive group and not as the potentially sensitive group themselves.

The current criterion just counts the number of MDD below specific values. So, increasing the number of samplings or prolongation of the study increases the chance to fulfil the criterion, especially for populations with MDD close to 100. It could be discussed if the MDD evaluation should be restricted to e.g. the period from the first application until eight weeks after the last application.

We invite others, especially regulators, to share their experience on the use of the MDD concept with us.

Brock T.C.M., et al. (2015): The minimum detectable difference (MDD) and the interpretation of treatment-related effects of pesticides in experimental ecosystems. Environmental Science and Pollution Research 22:1160–1174. (open access. DOI 10.1007/s11356-014-3398-2).
EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11(7):3290, 268 pp. doi:10.2903/j.efsa.2013.3290

General information				Statistical design					MDD category 1 taxa (genus or lowest available level)					MDD evaluation						
Nr.	Test system	year	Stressor	total n	Controls	Treatment levels	Replicates	Duration after 1st appl	Algae	Macrophytes	Crustacea	Insects	other Inv	Algae	Macrophytes	Crustaceans	Insects	Other inv	Sensitive MDD Cat 1 pop.	
1	5 m² outdoor mesocosms	2012	F	18	4	5	3 (2)	19	Chlamydomonas, Chroomonas Microcystis Nitzschia, Volvox		Daphnia, Chydorus, Cyclopoidae, Asellus , Crangonyx	Chaoborus, Cloeon, Orthocladiinae, Tanypodinae, Zygoptera	Keratella (2), Anuraeopsis, Helobdella, Erpobdella, Oligochaeta, Physa, Lymnaea , Musculium	5		5	5	9	24	✓
2	5 m² outdoor mesocosms	2013	F	18	4	5	3 (2)	16	Chlorophyceae, Diatomaeae		Daphnia, Cyclopoidae, Asellus , Crangonyx	Chaoborus, Cloeon, Chironomus, Polypedilum, Micropsectra, Paratanytarsus, Tanytarsus , Cricotopus , Procladius, Psectrotanypus, Zygoptera	Keratella (2), Oligochaeta, Helobdella, Erpobdella, Lymnaea, Radix, Physa, Musculium	2		4	11	9	26	✓
3	1.8 m³ outdoor enclosures	2014	F	15	5	5	2	14	Chlorella, Chromulina, Chroomonas, Chrysoph. (1-5 µ), Chrysoph (6-10 µ), Cryptomonas, Ochromonas, Trachelomonas		Daphnia, Cyclopidae, Asellus	Chaoborus, Coenagrionidae, Cloeon, Tanypodinae, Orthocladiinae	Acari, Polyarthra, Synchaeta, Keratella, Lymnaeidae, Dugesia, Lumbriculus, Helopdella	8		3	5	8	24	✓
4	1.8 m³ outdoor enclosures	2014	F	15	5	5	2	12	Ankyra, Trachelomonas, Chromulina, Chrysophyceae (1-5 µ), Chroomonas, Cryptomonas		Chydorus, Daphnia, Cyclopidae, Diaptomidae, Asellus	Chaoborus, Coenagrionidae, Cloeon, Tanypodinae	Keratella, Hexarthra, Planorbis, Dugesia	6		5	4	4	19	✓
5	1.8 m³ outdoor enclosures	2006	H	15	5	5	2	12	Ankyra , Characium , Chlamydomonas (2), Chlorella , Phacomyxa , Tetrachlorella, Rhodomonas, Cryptomonas, Chrysophyceae (15 µm), Chrysophyceae (610 µm), Trachelomonas	Ceratophyllum , Chara , Potamogeton (2), Stratiotes, Lemna				12	5				17	✓
6	1.8 m³ outdoor enclosures	2009	H	15	5	5	2	12	Chlorella, Chroomonas ., Chrysophyceae (15µm), Cryptomonas , Erkenia, Nephrochlamys, Nitzschia	Ceratophyllum , Chara , Potamogeton, Lemna				7	4				11	✓
7	5 m³ outdoor mesocosms	2010	H	13	3	5	2	8	Oedogonium, Nitzschia, Chroomonas, Characium	Myriophyllum, Potamogeton, Chara, Ceratophyllum, Sparganium, Lemna	Copepoda (Nauplia), Daphnia (2)	Chaoborus	Brachionus	4	6	2	3	1	10	✓
8	1.8 m³ outdoor enclosures	2012	H	16	4	6	2	8		Ceratophyllum, Chara , Glyceria , Mentha , Myriophyllum , Vallisneria, Lemna , Salvinia , Spirodela, Stratiotes					10				10	✓

General information				Statistical design					MDD category 1 taxa (genus or lowest available level)					MDD evaluation						
Nr.	Test system	year	Stressor	total n	Controls	Treatment levels	Replicates	Duration after 1st appl	Algae	Macrophytes	Crustacea	Insects	other Inv	Algae	Macrophytes	Crustaceans	Insects	Other inv	Sensitive MDD Cat 1 pop.	
9	6 m³ mesocosms	1999	I	12	3	5	2 (1)	91			Chydorus , Daphnia , Calanoida, Cyclopoida , Gammarus	Chironomus, Micropsectra, Bryophaenocladius , Procladius, Chaoborus, Cloeon	Stylaria, Helopdella (2), Asplanchna, Keratella, Polyarthra, Synchaeta			5	6	7	11	✓
10	5 m³ outdoor mesocosms	2004	I	13	3	5	2	13	Chlorophyceae, Chroomonas, Cryptomonas 1, Cryptomonas 2, Conjugatophyceae , Nitzschia		Asellus, Daphnia, Simocephalus, Chydorus, Cyclopoida, Ostracoda	Chaoborus, Chironominae, Psectrocladius, Holotanypus, Psectrotanypus	Stylaria, Gyraulus, Keratella, Polyarthra, Lepadella, Lecane, Synchaeta	6		6	5	7	11	✓
11	5 m³ outdoor mesocosms	2008	I	13	3	5	2	11	Chromulina, Characium, Chlamydomonas, Chroomonas, Cryptomonas, Gomphonema, Pennales		Daphnia, Simocephalus, Chydorus, Cyclopoida	Chaoborus, Psectrocladius, Cricotopus, Chironominae, Tanypodinae	Tubificidae, Helobdella, Brachionus, Keratella, Polyarthra, Mytilina	7		4	5	6	9	✓
16	1.8 m³ outdoor enclosures		I	15	5	5	2	10			Asellus, Chydorus, Daphnia, Diaptomidae, Cyclopidae, Gammarus (B)	Chaoborus, Cloeon, Chironominae, Tanypodinae, Zygoptera	Lymnaea, Planorbidae, Keratella, Polyarthra			6	5	4	11	✓
12	1.8 m³ outdoor enclosures	2014	I	15	5	5	2	10			Asellus, Chydorus, Daphnia, Diaptomidae, Cyclopidae, Gammarus (B)	Chaoborus, Cloeon, Chironominae, Tanypodinae, Orthocladiinae, Ceratopogonmidae, Heteroptera, Zygoptera	Lymnaea, Planorbidae, Tricladida, Keratella, Polyarthra, Trichotria			6	8	6	14	✓
13	1.8 m³ outdoor enclosures	2014	I	22	6	4	4	12		Ceratophyllum, Chara , Myriophyllum, Lemna	Asellus, Gammarus (B), Daphnia, Chydorus, Calanoidae, Cyclopidae	Cloeon, Chaoborus, Phryganea, Orthocladiinae, Tanypodinae, Anisoptera, Zygoptera	Keratella, Polyarthra, Dugesia, Lumbriculus, Naididae, Lymnaeidae		4	6	7	6	13	✓
15	5 m³ outdoor mesocosms	2015	I	18	6	4	3	10			Asellus, Crangonyx, Cyclopoida, Calanoida, Daphnia, Simocephalus	Chaoborus, Cloeon, Zygoptera, Culicidae, Chironomus, Micropsectra, Tanytarsus, Orthocladiinae, Tanypodinae, Ceratopogonidae	Oligochaeta, Helobdella, Erpobdella, Anuraeopsis, Keratella 1, Keratella2, Polyarthra, Lepadella, Radix, Lymnea, Physa			6	10	11	16	✓
16	1.8 m³ outdoor enclosures	2007	I	18	4	5	3 (2)	12			Argulus, Eudiaptomus, Cyclopoidae, Daphnia, Simocephalus, Asellus	Chaoborus, Chironomidae, Baetidae, Coleoptera	Keratella, Polyarthra, Synchaeta, Lymnaea			6	4	4	10	✓
17	1 m² microcosms in greenhouse	2002/ 2003	I	15	3	6	2	8			Daphnia (2), Chydorus, Cyclopoida, Ostracoda		Keratella (2), Cephalodella, Lepadella			5		4	5	
18	3 m² outdoor mesocosms	2014	Bio-cide	13	3	5	2	10			Daphnia (2), Cyclopoida, Asellus, Crangonyx	Chaoborus, Cloeon	Harringia, Keratella, Hexarthra, Lymnaea, Radix, Physa, Ologochaeta			5	2	7	14	✓
19	1 m² microcosms in greenhouse	2001	Cu	16	4	6	2		small Pennales, Rhodomonas, Ankyra	Egeria	Daphnia (2), Chydorus, Copepoda (Nauplia)		Keratella	3	1	4		1	9	✓
20	1 m² cosms in greenhouse	2009 / 2010	Ni	14	4	5	2	16	Ankyra, Chlorella, Chromulina, Chroomonas, Chrysophyceae (15µm), Coelastrum, Lyngbia, Monosiga, Nephrochlamys		Daphnia (2) Acroperus, Simocephalus		Lymnaea, Keratella (2), Brachionus, Hexarthra, Chephalodella	9		4		6	19	✓