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Scavenging amphipods and isopods – a novel marine resource?

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Background

✓ Large quantities of scavenging crustaceans destroy catch and eat the bait in fish pots and long-line fishing (Conlan 1994; Jákupsstovu et al. 2002).

✓ The proven dissemination and large quantities have led to question on the commercial value of these species as feed & food supplements.

✓ The fishing industry show interest for raw material with a putative commercial value.

Aims

✓ DEVELOP CATCH TECHNOLOGY FOR EFFICIENT SAMPLING OF SMALL, SCAVENGING CRUSTACEANS

✓ PERFORME INITIAL CHEMICAL & BIO-CHEMICAL PROFILING

Material & Methods

Development of novel pot technology
Performed in co-oporation with the Refa Frøystad Group (RFG) and Sanden Skjellprodukter. Different prototypes were tested in the Valderhaugfjord (Ålesund, Norway). Concluions were drawn after evaluation of triplicate experioments using 500 g mackerel as bait.

Chemical and biochemical profiling
Sampling: Whole specimens of the amphipod (*Tmetonyx cicada*) and the isopod (*Natatolana borealis)* collected between August - September 2010 were stored at – 40°C until analysis. Amphipods and isopods were screened for total protein, total lipids, metals (Zn, Cu, Cd, Fe, Hg, Pb, Sn,), brominated flame retardants (PBDEs, PBDFs) PCBs, dioxines and furanes using quality approved methods (EUROFINS, Amsterdam).

Fatty acid profiles
Lipids extracted according to Blight & Dyer (1959). Methylated fatty acids were analysed on a carbowax 20M-column on a Perkin Elmer GC equiped with a FID-detector.

Peptidase activity assay
2 g (~ 40 individuals) of T.cicacda were mixed with 2 vol ice-cold Na-phosphate buffer, pH 7 and homogenized with Ultra Thurrax for 2 min followed by centrufugation at 10000 x g for 60 min, at 4°C to pellet debris. The supernatant were used directly for detemination of peptidase activty using 1.5 % azocasein solution as substrate (Handbook of food analytical chemistry, Willey and Sons, 2005).
Enzyme reaction were performed at 30°C for 30 min. Protein concentrations were determined using the Bicinchoninic Acid Kit for Protein Determination (Sigma) using BSA as a standard. Enzyme activity were determined in triplicate. One unit (U) of total proteolytic activity was $\Delta A_{440\ nm}$/min cm.

Results

Novel catch technology developed

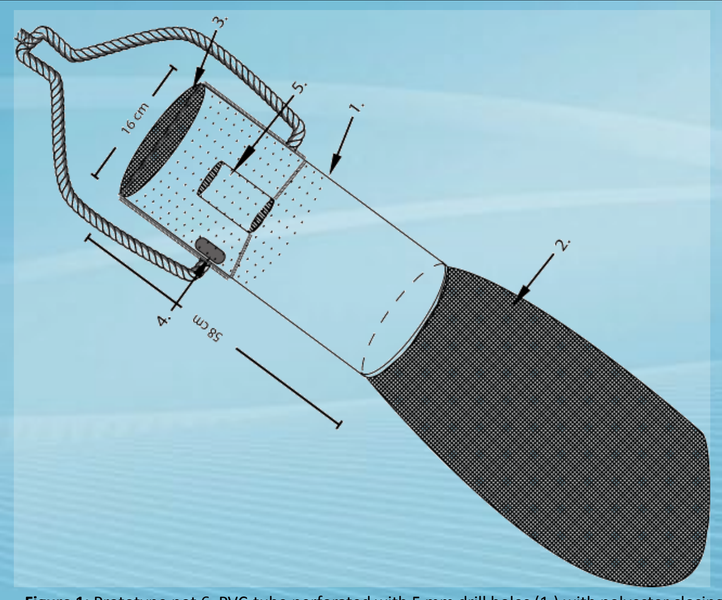


Figure 1: Prototype pot 6. PVC-tube perforated with 5 mm drill holes (1.) with polyester closing net at the bottom (200µm). The top of the pot consist of a screw-cap and a polyester closing net (200µm) (3.). The box containg bait is shown centered (5).

A prototype pot was developed (prototype#6) showing satisfactory results regarding quantity and quality. This prototype pot efficiently trapped isopods and amphipods in the range of 1- 10 kgs.

Figure 2: Pictures from field studies.

Chemical profiling

Fat, SBR	3.8	g/100 g	30%	NMKL 131	0.1
Protein					
Crude Protein Nx6.25	10.0	g/100 g	10%	NMKL 6	0.3

Table 1: Total lipid and protein in the amphipod *T.cicada*. The results are shown with uncertainty of measurments in %, method and limit of quantitation

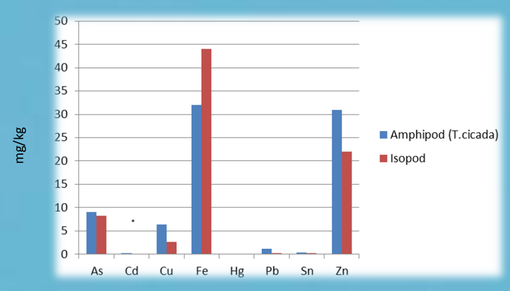


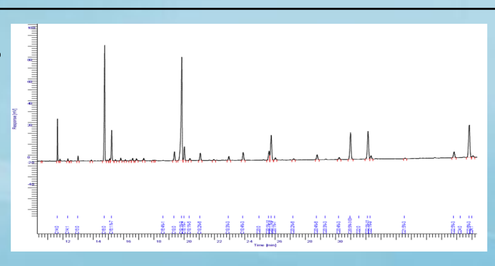
Figure 3. Heavy metal chemical profile of both species

All metals (exception Pb) are below allowed EU maximum limits for feed and food for those substances which maximum limits have been established. The allowed maximum limit for Pb is 0,2 mg/kg. The level of Pb probably reflect the geographical proximity to local industry and thus may not be representative for amphipods and isopods in general.

Results from the screening of brominated flame retardants (PBDEs, PBDFs) PCBs, dioxines and furanes indicate that leves are below the allowed maximum values for feed and food (data not shown). Noteworthy is that the Σ PCBs (PCB_7) is about 4.5 times higher (17 µg/kg) in *T.cicada* compared to the isopod *N.borealis* (4 µg/kg).

Fatty acid profiles

A.



B.

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Peak #	Component Name	Time [min]	Area [uV*sec]	Height [uV]	Area [%]
1		6,596	658,90	423,94	0,03
2	C14:0	7,640	75204,11	38778,15	3,65
3		7,798	3460,88	1791,64	0,17
4	C14:1	8,327	4808,96	2056,03	0,23
5		8,519	1334,73	580,62	0,06
6	C15:0	8,996	12010,80	4704,69	0,58
7		9,875	3253,22	989,77	0,16
8	C16:0	10,755	367832,79	106413,81	17,85
9		10,932	3258,61	871,31	0,16
10		11,083	7447,06	2017,67	0,36
11	C16:1 n-7	11,215	102056,12	28253,17	4,95
12		11,469	4700,72	1198,92	0,23
13		11,816	8834,14	2589,85	0,43
14		12,119	3627,10	1040,56	0,18
15		12,446	3164,73	783,57	0,15
16		12,602	9187,09	2377,87	0,45
17		12,850	7480,94	1824,00	0,36
18		13,333	8109,71	2156,60	0,39
19		13,949	1642,23	455,92	0,08
20		14,063	1288,15	439,08	0,06
21	C18:0	15,357	37047,38	8252,50	1,80
22	C18:1n-9	15,842	493388,72	95010,47	23,94
23	C18:1n-7	16,016	52129,60	12621,04	2,53
24	C18:1n-5	16,380	7988,98	1899,67	0,39
25	C18:2n-6	17,069	31864,84	6789,51	1,55
26		17,958	4635,12	906,48	0,22
27	C18:3n-3	18,955	17329,96	3651,44	0,84
28	C18:4n-3	19,896	35435,87	7255,71	1,72
29	C20:1n-11	21,604	44562,97	8405,18	2,16
30	C20:1 n-9	21,749	123885,07	22752,75	6,01
31	C20:1n-7	22,022	9769,05	2039,59	0,47
32	C20:2n-6	23,235	6170,29	1112,08	0,30
33	C20:4n-6	24,784	25013,87	4499,21	1,21
34	C20:4n-3	26,233	10266,25	1898,10	0,50
35	C20:5n-3 EPA	26,983	135022,77	24595,28	6,55
36	C22:1n-11	28,141	147995,22	25424,74	7,18
37	C22:1n-9	28,345	16855,28	3110,06	0,82
38	C21:5n-3	30,583	3824,78	678,99	0,19
39	C22:5n-3	33,826	31563,40	5320,18	1,53
40	C22:6n-3	34,829	188839,63	29567,30	9,16
41	C24:1	35,073	7868,84	1284,10	0,38

Chromatogram (A) and table (B) showing results from GC-analysis of methylated fatty acids in *T.cicada.* C16:0 and C18:1 fatty acids (omega-9) constitute about 40 % of total lipids. Omega-3 fatty acids such as EPA, and DHA constitute about 15 % of total lipids .

Total proteolytic activity

The crustaceans extracts showed a protein content of 20 ± 2 mg/ml. At pH 7 and 30°C the proteolytic specific activity was determined to be 0.016 ± 0.05 U/min cm.

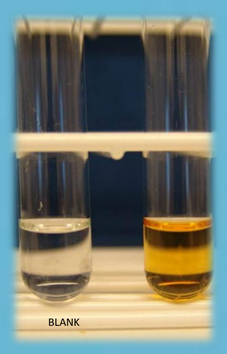

Figure 5: Demonstration of proteolytic activity in crude protein extracts from *T.cicada* determined by hydrolysis of azocasein. Inactivated crude protein extracts were used as blanks.

Conclusions

✓ Possible to capture larger quantities of amphipods and isopopods using pot-technology developed by Møreforsking Marin.
✓ Low levels of undesirable substances. Local pollution probably contribute to undesired Pb levels.
✓ High protolytic activity suggest interesting bioactivity properties.

Future work

Continue screening of bioactivity:
✓ Enzymatic:
- Inhibition studies of proteolytic activity.
- Screen for novel lipases.

✓ Screen for bioactive lipids and peptides.

MØREFORSKING

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Isopod (*Natatolana borealis*) Foto: Snorre Bakke


Sorting of isopods and amphipods. Foto: Møreforsking Marin


N. borealis with and without gut content Foto: Møreforsking Marin


Amphipod (T.cicada) Foto: Snorre Bakke