THE CONCENTRATION OF MERCURY, COPPER, NICKEL, SILVER, CADMIUM, AND LEAD IN THE NORTHERN ADRIATIC ANCHOVY, *ENGRAULIS ENCRASICHOLUS*, AND SARDINE, *SARDINA PILCHARDUS*

MALVERN GILMARTIN[1] AND NOELIA REVELANTE[2]

ABSTRACT

Levels of mercury, copper, nickel, silver, cadmium, and lead were determined in various tissues of the northern Adriatic anchovy, *Engraulis encrasicholus*, and sardine, *Sardina pilchardus*, throughout a 7-month fishing season. The highest concentrations of nickel, silver, cadmium, and lead occurred in the skin and gills, with little interspecific differences and no unusually high values. The highest concentrations of mercury and copper occurred in internal tissues, with the anchovy showing markedly higher concentrations than the sardine. Total mercury concentrations in anchovy muscle ranged from 70 to 215 nanograms per gram wet weight, concentrations 2-4× greater than in the same or similar anchovy species off northwestern Africa, southeastern United States, and California.

The Adriatic Sea extends 800 km into the heartland of the European continent, and is bordered by Italy, Yugoslavia, and Albania. The extended coastal shelf of the northern portion is an important fishery region, exploited heavily by both Italy and Yugoslavia.

Unfortunately, with restricted exchange to the open Mediterranean, the Adriatic displays many of the characteristics of a narrow trapped sea. Furthermore, the shallow northern region receives industrial wastes disproportionate to its area from large industrial centers located along the eastern coast at Rijeka and Pula, Yugoslavia, and more extensive industrial concentrations on the Gulf of Trieste and the western coast near Venice, Italy. More significantly, the Reno, Po, Adige, and Isonzo rivers discharge their industrial pollutant loads into the northern Adriatic. The Po alone is the second largest and perhaps most heavily polluted river entering the entire Mediterranean, and has a drainage area more than three-quarters the total area of the Adriatic itself. In 1972, 76 plants were discharging their waste water directly or indirectly into the Venice lagoon, and 10,000 of the 64,000 hectares of the lagoon were considered seriously polluted. Consequently, it is estimated that about 70% of the total organic load of industrial waste entering the Adriatic Sea from the west is concentrated in the northern part of the sea (General Fisheries Council for the Mediterranean 1972).

In contrast, although the deeply indented eastern coast has more than twice the shoreline of the western coast, population density and industrialization are markedly lower, and no major rivers flow into the Adriatic from the east. However, the level of industrialization and population density is increasing on both coasts of the Adriatic, and already the level of some heavy metals (e.g. mercury) in water and edible marine organisms in the open Adriatic may be at, or even slightly above, the acceptable safety level (General Fisheries Council for the Mediterranean 1972).

Recent research has drawn attention to the increasing hazards of heavy metal pollution in the marine environment (Ruivo 1972). Continental shelf fisheries, such as those of the shallow northern Adriatic, are especially threatened by these materials since large amounts can be introduced into restricted areas by coastal waste water runoff and the fallout of aerosols.

Therefore, a preliminary survey of the levels of mercury and copper, and the potentially toxic metals nickel, silver, cadmium, and lead in the sardine *Sardina pilchardus* Walbaum and the

[1]Australian Institute of Marine Sciences, P.O. Box 1104, Townsville, Queensland 4810, Australia.
[2]Center for Marine Research, Institute "Rudjer Boskovic," 52210 Rovinj, Yugoslavia.

Manuscript accepted May 1974.

anchovy *Engraulis encrasicholus* Linnaeus was conducted during 1972 and 1973 to establish currently occurring levels of these elements and to produce a baseline for future reference.

The Fishery

The total fish catch landed in the eastern Adriatic remained relatively stable from 1964 through 1971, at 25,000-30,000 metric tons (Food and Agriculture Organization of the United Nations 1972), with an additional 2,000-3,000 metric tons taken annually for noncommercial use. Three small pelagic species of clupeids consistently compose at least 70% of this catch (Table 1), about 85% of which is canned, the balance being sold fresh. No significant fish meal industry currently utilizes these species.

There is strong evidence that the sardine is already fished to capacity, but that the anchovy may be significantly underexploited (Major 1970). The two species selected for the initial survey were the sardine, the most abundant and commercially most important species in the Adriatic, and the anchovy, the species suspected of having the greatest potential for increased production.

The Stock

The population structure and migration patterns of the Adriatic sardine and anchovy are not well understood. Gamulin (1956) opined that the sardine population migrates offshore annually from the very shallow waters of the northern Adriatic to somewhat deeper waters (60-120 m), but not into the central Adriatic, a behavior pattern typical of the species (Larrañeta 1960). Zavodnik (1968) presented similar evidence, noting that the species is completely absent from the shallow coastal zone by December. Krajnović and Dekaris (1968) found serological evidence that various subpopulations pass the Istrian coast during these migrations.

Evidence for anchovy migrations, e.g. based on seasonal variations in catch statistics at various ports, appears somewhat clearer. Piccinetti (1970) suggests that the population "winters" off Ancona and farther south off the western coast of the Adriatic, and during the spring migrates eastward and then northward along the Dalmatian coast, reaching the Istrian islands and peninsula during early summer. Continuing into the Gulf of Trieste and westward to Venice during autumn, the population returns to its "wintering" grounds by December.

While it is difficult to define exact migratory routes, or to delimit subpopulations, the evidence suggests that a composite stock of sardine and anchovy exists in the northern Adriatic, and that the Rovinj fishery "samples" much of this stock as it migrates past the Yugoslav Istrian peninsula.

MATERIALS AND METHODS

Sampling

During 1972 the major fishing season off Istria extended from June to December, rather than the more common May to October season. Samples were collected throughout the 1972 season and during the first few months of the 1973 season from the catch landed at the Mirna Cannery in Rovinj, Yugoslavia. These fish were usually caught within the preceding 12-18 h, within 15-30 km of Rovinj, using night light and purse seine.

Each sample usually consisted of at least six "representative" sardines and six "representative" anchovies selected from the catch, although often 50, and occasionally up to 60, fish were collected. Samples were immediately taken to

TABLE 1.—Yugoslavia's marine fisheries catch, 1964-71.

Species[1]	Nominal catch in thousands of metric tons							
	1964	1965	1966	1967	1968	1969	1970	1971
Sardina pilchardus (sardine)	5.4	9.4	9.1	11.2	13.5	13.5	10.9	14.7
Clupea sprattus (sprat)	5.0	5.7	3.4	2.5	3.2	2.0	5.5	3.8
Engraulis enchrasicholus (anchovy)	4.9	2.2	4.8	7.3	4.8	4.0	3.0	3.1
Scomber scombrus (Atlantic mackerel)	2.6	0.8	2.3	1.2	1.1	0.5	0.3	0.1
Boops boops (greater amberjack)	1.3	0.7	1.4	1.4	1.4	1.3	1.1	1.0
Maena maena, M. smaris (mullet)	1.3	1.1	1.0	1.0	0.9	0.8	0.7	0.3
Misc. marine fishes (18 spp.), Crustacea, and Mollusca	4.8	6.1	5.3	5.4	5.0	4.8	4.1	7.8
Total marine catch	25.3	26.0	27.3	30.0	29.9	26.9	25.6	30.8

[1]All scientific and common names from Bini (1965).

the Center for Marine Research in Rovinj, weighed and their standard length determined. Each fish was then dissected into component parts, e.g. skin (including scales), gills, muscle. These components were then pooled according to type, weighed, dried to a constant weight at 110°C, ground to homogeneous meal in a porcelain mortar, and stored in a glass desiccator until analyzed.

Analytical Methods

Standard chemical procedures (Christian and Feldman 1970; Fletcher 1970; Uthe et al. 1970; Stainton 1971) as modified by Knauer and Martin (1972) were used. For all elements except mercury, two 1-g aliquots of sample plus 10 ml 70% redistilled HNO_3 were added to 30-ml beakers, which were covered with watch glasses and refluxed at 90°C for 2 h, followed by evaporation to dryness. After charring, the samples were redissolved in 5 ml 70% HNO_3, followed by the dropwise addition of 30% H_2O_2 until the samples remained clear. At this point the samples were evaporated to 5 ml, cooled, and diluted to 25 ml with distilled water.

All samples were analyzed on a Perkin-Elmer 303 atomic absorption spectrophotometer.[3] Various combinations of fuels and oxidants, as recommended in the Perkin-Elmer manual, were used for each element. Small differences between signal and base line in some of the Cd, Ni, and Ag analyses may have introduced error.

Procedures for mercury differed slightly. Approximately 0.2-0.4 g dry weight of sardine and anchovy tissues were weighed into 20-ml Neutraglas disposable ampoules, followed by the addition of 3 ml of a 2:1 solution of concentrated H_2SO_4 and HNO_3. Samples were heated on a hotplate overnight at 80°C, cooled in ice, followed by the addition of 8 ml 6% $KMnO_4$ until the solution just turned pink. Subsequently 2 ml of sample digest were drawn into a 10-ml disposable syringe, followed by the addition of 2 ml reductant, and partitioning. The mercury was partitioned between liquid and air on a vortex mixer by drawing the syringe back to 10 ml (leaving a 6-ml air space). The vapor was then injected into a specially constructed 3-ml (total volume) glass absorption cell which had been mounted on the burner assembly of a Perkin-Elmer 303 atomic absorption spectrophotometer. A Westinghouse hollow cathode mercury lamp was used as an energy source. Three sets of blanks and three sets of standards consisting of 10, 20, and 30 ng mercury ($HgCl_2$) in triplicate were interspersed within a given sample run.

[3]Reference to trade names does not imply endorsement by the National Fisheries Service, NOAA.

Calibration

During the analyses, aliquots of National Bureau of Standards Orchard Leaves No. 1571 were routinely analyzed for each element (Table 2). Similar standardizations conducted by Knauer (1972) during his study of metals in the northern anchovy, *Engraulis mordax*, and an interlaboratory calibration using both the destructive analytical techniques with atomic absorption spectrophotometry and non-destructive activation analysis (Knauer 1972; Goldberg 1972), indicate that our standardizations compare well with other laboratory analyses for these elements (Table 2).

TABLE 2.—Comparative standardizations of elemental concentrations in National Bureau of Standards Orchard Leaves No. 1571 in micrograms per gram (Hg in nanograms per gram).

Laboratory	Hg $\bar{x}$ C.V.	Cu $\bar{x}$ C.V.	Ni $\bar{x}$ C.V.	Ag $\bar{x}$ C.V.	Cd $\bar{x}$ C.V.	Pb $\bar{x}$ C.V.
National Bureau of Standards	155 ± 15	12.0 ± 0.3	1.3 ± 0.2		0.11 ± 0.02	45.0 ± 3.0
Hopkins Marine Station[1]						
Knauer	160 ± 17	12.0 ± 1.0	0.6 ± 0.3	<0.05	0.21 ± 0.1	41.0 ± 3.8
Gilmartin and Revelante	166 ± 22	10.0 ± 1.0	2.3 ± 0.4	0.15 ± 0.04	0.30 ± 0.1	41.0 ± 4.3
Skidaway[1]	150 ± 30	9.9 ± 0.5			0.32 ± 0.06	40.9 ± 3.3
Battelle-Northwest[2]	180 ± 20	11.2 ± 1.3		<0.02	0.23 ± 0.06	
Puerto Rico Nuclear Center[2]	170 ± 50	11.8				[1]50.0

[1]Flameless atomic absorption spectroscopy.
[2]Neutron activation analysis.

RESULTS

The six metals studied are considered from the viewpoint of differences in concentrations between the two species, variable concentrations in different tissues, and seasonal variations. Results are expressed in $\mu g\ g^{-1}$ wet weight for all elements except mercury, which is expressed in $ng\ g^{-1}$ wet weight. These can be converted to dry weight using the factors presented in Table 3.

Mercury

The seasonal distribution of mercury in various tissues in both the sardine and anchovy ranged from 5 to 610 $ng\ g^{-1}$ wet weight (Table 4). During

TABLE 3.—Sample size, length data, and wet:dry ratio for sardine and anchovy tissues analyzed for metal concentrations.

		Length (cm)		Wet:dry weight ratios							
Date	*n*	Mean	Range	Skin	Gills	Muscle	Digest.	Liver	Kidney	Gonad	Brain
Sardine:											
9 June 72	6			2.18	3.81	3.79	3.90	2.80			
10 July 72	6	12.90	12.7-13.2	1.38	4.57	3.71	3.22	3.56			
17 Aug. 72	6	16.14	15.5-16.5	2.36	4.62	4.04	4.48	3.47			
29 Sept. 72	5	15.28	14.6-16.0	1.53	4.30	3.54	2.43	3.76			
31 Oct. 72	6	17.85	7.0-18.8	1.56	4.36	3.55	2.33	4.64			
5 Dec. 72	6	15.08	14.4-16.0	1.82	4.14	3.86	4.00	1.67			
28 Mar. 73	6	17.69	16.7-18.3	1.88	5.31	3.94	4.45	4.04			
4 May 73	16	15.29	14.7-16.0	1.87	4.34	3.90	4.49	3.58	3.20	3.78	
8 May 73	51	15.39	14.1-17.4	1.81	4.44	4.10	4.58	3.47	2.87	3.99	3.94
24 May 73	49	14.93	14.1-15.8	2.05	4.09	3.88	4.40	3.41	3.78	4.69	4.23
6 June 73	45	15.54	14.6-16.6	2.23	4.17	3.70	3.54	3.55	3.51	3.69	4.04
Mean				1.88	4.38	3.82	3.80	3.45	3.34	4.04	4.07
Anchovy:											
9 June 72	6			1.73	3.92	3.47	2.65	2.56			
10 July 72	6	12.67	12.4-13.0	1.68	4.96	3.74	2.22				
17 Aug. 72	6	14.50	13.6-15.8	1.34	4.12	3.12	1.96	2.81			
3 Oct. 72	6	13.98	13.3-14.8	1.30	4.75	3.23	2.06	3.32			
31 Oct. 72	6	14.52	14.2-14.8	1.50	4.86	2.97	2.58	3.99			
6 Dec. 72	6	14.43	14.2-14.6	1.36	5.64	3.49	3.33	3.70		4.72	
29 Mar. 73	6	14.40	14.1-14.6	1.87	6.92	4.53	3.41	3.84			
24 May 73	60	13.37	12.2-14.8	1.81	3.70	3.89	4.16	3.35	3.19	4.09	3.81
6 June 73	52	14.75	13.2-16.6	2.52	4.10	4.03	5.21	3.46	4.32	3.56	4.06
Mean				1.68	4.77	3.61	3.06	3.38	3.76	4.12	3.94

TABLE 4.—Concentrations of total mercury ($ng\ g^{-1}$ wet weight) in tissues of sardine *Sardina pilchardus* and anchovy *Engraulis encrasicholus* from the Adriatic Sea.

Date	Skin[1]	Gills	Muscle	Digest.[1]	Liver	Kidney	Gonads	Brain	Tot. fish
Sardine:									
9 June 72	70		105	ND					80
10 July 72	35		40	ND					30
17 Aug. 72	10		135	90					110
29 Sept. 72	25		95	60					75
31 Oct. 72	35		115	110					105
5 Dec. 72	5		85	80					65
28 Mar. 73	105	25	115	100	155				100
4 May 73	30	35	80	80	160	260	20	15	60
8 May 73	60	35	90	115	215	455	40	35	85
24 May 73	45	30	95	60	210	220	30	35	80
6 June 73	70	45	120	115	210	330	75	35	165
Mean	45	35	100	75	190	315	40	30	85
Anchovy:									
9 June 72	75		120	ND					105
10 July 72	40		75	125					75
17 Aug. 72	55		215	ND					190
3 Oct. 72	ND		165	ND					140
31 Oct. 72	200		155	ND					160
6 Dec. 72	190		215	185					165
29 Mar. 73	70	25	85	140	215	295			65
24 May 73	80	45	70	110	255	370	35	25	75
6 June 73	145	60	175	215	415	610	80	35	170
Mean	95	45	140	85	295	425	60	30	125

[1]ND = not detected.

the early part of the study period, insufficient material was available to determine levels in certain tissues, but very significant species differences were noted between fish collected at the same point in time. In most instances mercury was present in greater amounts in specific anchovy tissues and the mean whole fish concentration was about 50% higher in anchovies.

The highest concentrations of mercury were noted in the liver and kidneys. On an annual mean basis the skin and gills of the sardine contained relatively low levels of mercury relative to other body tissues. In contrast, anchovy skin contained higher levels, especially during winter months when concentrations of 190-200 ng g^{-1} wet weight were observed. The greatest seasonal variation occurred in the digestive tract, where mercury ranged from not detected to 215 ng g^{-1}.

Copper

Copper levels ranged from 0.6 to 8.5 $\mu g\ g^{-1}$ wet weight (Table 5). As with mercury, anchovy tissues often contained higher concentrations of copper than corresponding sardine tissues, although the differences between the two species were not nearly so pronounced. The highest concentrations were observed in the liver of both species, with the skin of the anchovy consistently having significantly higher concentrations of copper than that of the sardine. On occasion the digestive tract of the anchovy contained up to 5.4× the concentrations in comparable sardine samples.

TABLE 5.—Concentrations of copper ($\mu g\ g^{-1}$ wet weight) in tissues of sardine and anchovy from the Adriatic Sea.

Date	Skin	Gills	Muscle	Digest.	Liver	Total fish
Sardine:						
9 June 72	1.8	1.0	0.9	1.4	3.7	1.09
10 July 72	1.5	0.9	1.2	1.5	—	1.05
17 Aug. 72	1.2	0.6	0.9	1.0	2.5	0.96
29 Sept. 72	1.2	0.7	1.1	0.8	3.4	0.94
31 Oct. 72	0.9	0.7	0.9	1.4	2.1	0.97
5 Dec. 72	1.9	1.1	1.0	1.7	3.5	0.98
Mean	1.4	0.8	1.0	1.3	3.0	1.0
Anchovy:						
9 June 72	1.8	0.8	0.7	1.7	4.0	1.05
10 July 72	2.0	0.9	0.6	1.9	2.8	0.96
17 Aug. 72	3.1	0.8	0.8	2.9	3.9	1.09
3 Oct. 72	[1]17.5	0.8	0.8	4.3	3.2	1.52
31 Oct. 72	2.8	0.8	0.8	3.3	1.0	1.14
6 Dec. 72	2.5	0.6	0.6	1.5	8.5	0.98
Mean	2.4	0.8	0.7	2.6	3.9	1.1

[1]Suspect value — excluded from mean.

Nickel and Silver

In contrast with mercury and copper, the concentrations of nickel and silver rarely showed significant differences between the two species. Nickel was consistently detected in muscle tissue whereas silver was not, and nickel was occasionally observed in anchovy livers but not sardine (Tables 6, 7). Although data indicate higher concentrations in the gills and skin, these may be artifacts introduced by scatter caused by bone matrix in the gills and clays in the skin. The concentrations reported are therefore considered maximal estimates.

TABLE 6.—Concentrations of nickel[1] (μg^{-1} wet weight) in tissues of sardine and anchovy from the Adriatic Sea.

Date	Skin	Gills	Muscle[2]	Digest.	Liver[2]	Total fish
Sardine:						
9 June 72	1.3	0.9	ND	0.3	ND	0.54
10 July 72	2.7	0.8	0.5	0.3	ND	0.53
17 Aug. 72	2.2	0.5	ND	0.1	ND	0.56
29 Sept. 72	2.9	0.5	0.3	1.0	ND	0.56
31 Oct. 72	1.5	0.7	0.3	ND	ND	0.59
5 Dec. 72	4.5	1.0	ND	0.6	ND	0.59
Mean	2.5	0.7	0.2	0.4	ND	0.6
Anchovy:						
9 June 72	1.7	0.9	0.3	0.4	ND	0.56
10 July 72	1.7	0.6	0.4	1.7	0.3	0.74
17 Aug. 72	1.1	0.2	0.3	0.6	0.2	0.58
3 Oct. 72	1.4	0.8	0.3	0.3	ND	0.46
31 Oct. 72	3.4	0.7	0.2	0.6	1.1	0.64
6 Dec. 72	5.8	0.9	0.2	0.9	ND	0.85
Mean	2.5	0.7	0.3	0.8	0.3	0.6

[1] = maximal estimates due to matrix problem.
[2]ND = not detected.

TABLE 7.—Concentrations of silver[1] ($\mu g\ g^{-1}$ wet weight) in tissues of sardine and anchovy from the Adriatic Sea.

Date	Skin[2]	Gills	Muscle[2]	Digest.[2]	Liver[2]	Total fish
Sardine:						
9 June 72	0.3	0.1	ND	ND	ND	<0.09
10 July 72	0.3	0.1	ND	ND	ND	<0.08
17 Aug. 72	0.4	0.2	ND	ND	ND	<0.10
29 Sept. 72	0.6	0.1	ND	ND	ND	<0.10
31 Oct. 72	0.2	0.2	ND	ND	ND	<0.09
5 Dec. 72	0.7	0.2	ND	<0.1	ND	0.10
Mean	0.4	0.2	ND	ND	ND	0.1
Anchovy:						
9 June 72	0.4	0.1	ND	ND	ND	<0.08
10 July 72	0.5	0.1	ND	ND	ND	<0.08
17 Aug. 72	0.4	0.2	ND	ND	ND	0.08
3 Oct. 72	0.2	0.2	ND	ND	ND	0.09
31 Oct. 72	ND	0.2	ND	ND	ND	<0.07
6 Dec. 72	0.5	0.1	ND	<0.1	ND	<0.10
Mean	0.3	0.2	ND	ND	ND	0.1

[1] = maximal estimates due to matrix problem.
[2]ND = not detected.

Cadmium

Concentrations of cadmium in various anchovy and sardine tissues ranged from less than 0.1 to 1.4 $\mu g\ g^{-1}$ wet weight (Table 8). No significant difference was observed between the two species, except for the anchovy liver which had consistently higher levels than all other tissues. Higher concentrations occurred in the skin of both species.

Lead

Lead concentrations in sardine tissues paralleled those in the anchovy (Table 9). High concentrations occurred in the gills and skin of both species, and a tendency for higher levels in anchovy liver late in the year was observed. The lowest values tended to occur in muscle tissue, with seasonal means ranging from not detected to 1.2 $\mu g\ g^{-1}$ wet weight.

TABLE 8.—Concentrations of cadmium ($\mu g\ g^{-1}$ wet weight) in tissues of sardine and anchovy from the Adriatic Sea.

Date	Skin	Gills	Muscle[1]	Digest.[1]	Liver[1]	Total fish
Sardine:						
9 June 72	0.2	0.2	<0.1	0.1	0.2	0.11
10 July 72	0.3	0.2	<0.1	0.3	ND	0.11
17 Aug. 72	0.4	0.1	ND	ND	0.4	0.10
29 Sept. 72	0.5	0.1	ND	ND	0.2	0.09
31 Oct. 72	0.3	0.1	ND	0.1	0.1	0.10
5 Dec. 72	0.5	0.2	ND	0.3	0.1	0.11
Mean	0.4	0.2	<0.1	0.1	0.2	0.1
Anchovy:						
9 June 72	0.4	0.2	<0.1	0.2	0.9	0.16
10 July 72	0.3	0.3	0.1	0.4	0.5	0.13
17 Aug. 72	0.5	0.3	<0.1	0.2	0.7	0.13
3 Oct. 72	0.2	0.1	ND	0.2	0.5	0.09
31 Oct. 72	0.4	0.2	<0.1	0.2	ND	0.12
6 Dec. 72	0.6	0.1	<0.1	0.2	1.4	0.20
Mean	0.4	0.2	<0.1	0.2	0.7	0.1

[1]ND = not detected.

TABLE 9.—Concentrations of lead ($\mu g\ g^{-1}$ wet weight) in tissues of sardine and anchovy from the Adriatic Sea.

Date	Skin	Gills	Muscle[1]	Digest.[1]	Liver[1]	Total fish
Sardine:						
9 June 72	4.5	3.0	0.4	ND	ND	1.23
10 July 72	4.9	4.5	0.1	ND	ND	0.94
17 Aug. 72	4.3	2.6	ND	ND	ND	0.82
29 Sept. 72	5.3	1.9	ND	2.2	ND	0.84
31 Oct. 72	3.3	2.4	ND	0.7	ND	1.38
5 Dec. 72	6.8	3.1	ND	0.3	ND	1.40
Mean	4.9	2.9	0.1	0.5	ND	1.1
Anchovy:						
9 June 72	2.7	4.3	ND	ND	ND	0.99
10 July 72	5.2	3.7	ND	0.4	ND	0.99
17 Aug. 72	5.7	3.9	ND	0.3	1.1	0.51
3 Oct. 72	1.6	2.6	ND	1.3	1.5	0.73
31 Oct. 72	7.0	3.1	1.2	0.7	3.4	1.16
6 Dec. 72	6.5	3.9	0.3	0.4	[2]11.0	1.37
Mean	4.8	3.6	0.3	0.5	1.2	1.0

[1]ND = not detected.
[2]Suspect value — excluded from mean.

DISCUSSION

The six heavy metals considered during this study fall into two groups with regard to distribution in tissues and differences in concentrations between the two species: nickel, silver, cadmium, and lead, with no major interspecific differences and the highest concentrations occurring in the skin and gills, and mercury and copper, with marked interspecific differences and the highest concentrations occurring in internal tissues.

The highest concentrations of nickel, silver, cadmium, and lead were observed in the skin and gills, perhaps associated with adsorption. A significant interspecific difference was observed with nickel and lead, with these elements being nondetectable in the sardine liver but frequently being detected in anchovy liver, suggesting a difference in feeding habits and/or migration routes of the two species.

A tendency for increasing lead concentrations in the skin during winter was evident, related perhaps to wind-induced roiling of sediments into the water column during this period. The northern Adriatic is known to have relatively high concentrations of metal pollutants in the bottom sediments (Selli et al. 1972; Štirn pers. commun. 1973). A comparison of the concentrations of these metals in various tissues of the Adriatic and northern anchovies (Table 10) indicated that both nickel and cadmium occurred at approximately the same level. The relatively high nickel concentrations reported for the skin of the Adriatic anchovy could be an artifact introduced by analytical techniques (Table 2).

Copper and mercury showed a very different distribution pattern, with concentrations highest in the digestive tract and liver of the two clupeids, and seasonal means 2 to 3× greater than in the skin and gills, suggesting that ingestion may be the primary entry route into the organism. Concentrations in anchovy muscle tissue were markedly higher than in the sardine, contrasting with Establier's (1972) report that there were no differences in mercury concentration between these species off northwest Africa.

TABLE 10.—Mean elemental concentrations in *Engraulis mordax* collected from Monterey Bay, Calif. (Pacific) from Knauer (1972) and in *E. encrasicholus* from Rovinj, Yugoslavia (Adriatic) (mercury: ng g^{-1} wet weight: other elements: $\mu g\ g^{-1}$ wet weight).

Element	Location	Skin	Gills	Muscle	Digest.	Liver	Gonads
Mercury	Pacific	10	30	40		90	15
	Adriatic	95	45	140		295	55
Copper	Pacific	1.6	3	1.6	5	3	2
	Adriatic	2.4	0.8	0.7	2.6	3.9	
Nickel	Pacific	0.4	0.7	ND	1.1	ND	ND
	Adriatic	2.5	0.7	0.3	0.8	0.3	
Silver	Pacific	0.2	0.4	0.01	0.07	ND	0.4
	Adriatic	0.3	0.2	ND	ND	ND	
Cadmium	Pacific	0.2	0.3	0.03	2.8	1	0.3
	Adriatic	0.4	0.2	<0.1	0.2	0.7	
Lead	Pacific	3	4.8	0.2	1.2	ND	0.2
	Adriatic	4.8	3.6	0.3	0.5	1.2	

ND = not detected.

The calculated concentrations of mercury in the whole sardine range from 100 to 645 ng g^{-1} dry weight, with a seasonal mean of 320. These values compare favorably with the 580 ng g^{-1} dry weight reported by Ui (1971) for sardine collected in coastal waters 2 to 3 km north of Porto Corsini, on the Italian side of the northern Adriatic. Levels of mercury found in the northern Adriatic anchovy were relatively high when compared with levels in the same or similar species in other parts of the world. Mean concentrations of 140 ng g^{-1} wet weight were found in muscle tissue, and values in excess of 200 ng g^{-1} were observed (Table 4). The same species from the Gulf of Cádiz and off northwestern Africa contained 60 and 50 ng g^{-1} wet weight respectively (Establier 1972); and the northern anchovy, *Engraulis mordax*, occupying the same ecological niche (see Baxter 1967), contained concentrations in muscle tissue averaging 40 ng g^{-1} wet weight (Knauer and Martin 1972). These last data are directly comparable, since the analyses were conducted in the same laboratory using the same techniques and equipment.

Short-lived, low trophic level fish such as the anchovy have a tendency to concentrate low levels of mercury relative to longer-lived higher order carnivores, e.g. tunas and billfishes (Rivers et al. 1972), yet the higher concentrations of mercury in internal tissues indicate an accumulation in the Adriatic anchovy as well. However, it should be noted that the source of such mercury is not necessarily lower trophic level organisms. On entering the sea, mercury is immediately bound to proteinaceous materials, and if not adsorbed or absorbed by organisms, is often present in detritus/matrixlike suspended matter (see Keckes and Miettinen 1972, for review). Since massive amounts of suspended matter are found in feeding areas, filter feeders cannot avoid ingesting it (Leong and O'Connell 1969).

While mercury input to the sea results from a combination of natural processes, such as weathering and atmospheric fallout, and from various anthropogenic processes, a number of recent studies have focused on the pollution resulting from increasing urbanization and industrialization along the northern Adriatic coast, in particular within the Po River watershed (Štirn 1965, 1970; Majori, Morelli, Diana, and Rausa 1967; Majori, Rausa, Morelli, and Diana 1967; Majori et al. 1968; Majori 1968; Panella 1968a, b, 1970; Cescon and Grancini 1971; General Fisheries Council for the Mediterranean 1972). Three factories in northern Italy use the same acetaldehyde process as the Japanese Minimata plant (Ui and Kitamura 1971), and two of these are located on the coast at Ravenna and Venice, offshore of which are the spring spawning grounds of the anchovy (Štirn 1969). In addition, the wintering grounds of the anchovy lie to the south, in an area influenced by the Po River (Štirn 1969; Piccinetti 1970).

The rate of mercury elimination by fish is slow, e.g. 267-700 days (Keckes and Miettinen 1972). This time scale easily spans annual migrations encompassing the northern Adriatic and emphasizes the necessity for international consideration of the potential pollution of the region. Although the levels of mercury observed in this study are well within the tolerances established by most countries (e.g. 200 ng g^{-1} muscle wet weight in Switzerland, 500 ng g^{-1} in the United States, and 1,000 ng g^{-1} in Italy and Japan), it is likely that with increasing anthropogenic input to the northern Adriatic, concentrations will increase, perhaps to prohibitory levels. Thus it is essential that monitoring continue, especially of the clupeid *Engraulis encrasicholus*, which not only concentrates mercury to a greater degree than the sardine, but which may represent one of the few significantly underutilized fish resources of the northern Adriatic Sea.

ACKNOWLEDGMENTS

Support for this study was provided through U.S. National Science Foundation grants GF 31947X and GA 31271X. The authors gratefully acknowledge the technical assistance of Anica Cerin and Keith Skaug.

LITERATURE CITED

BAXTER, J. L. (editor).
1967. Symposium on anchovies, genus *Engraulis*. Calif. Coop. Oceanic Fish. Invest., Rep. 11:27-139.

BINI, G.
1965. Catalogo dei nomi dei pesci dei molluschi e dei crostacei di importanza commerciale nel mediterraneo (Catalogue of the names of commercially important fish, molluscs, and crustaceans in the Mediterranean Sea). Gen. Fish. Counc. Mediterr., FAO (Food Agric. Organ. U.N.), Rome, 407 p.

CESCON, B., AND G. GRANCINI.
1971. On some aspects of the marine pollution in Venice lagoon. Boll. Geofis. Teor. Appl. 13(49):36-43.

CHRISTIAN, G. D., AND F. J. FELDMAN.
1970. Atomic absorption spectroscopy; applications in agriculture, biology, and medicine. Wiley-Intersci., N.Y., 490 p.

ESTABLIER, R.
1972. Concentración de mercurio en los tejidos de algunos peces, moluscos y crustáceos del Golfo de Cádiz y caladeros del noroeste africano. Invest. Pesq. 36:355-364.

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS.
1972. Catches and landings, 1971. FAO, Yearb. Fish. Stat. 32, 558 p.

FLETCHER, K.
1970. Some applications of background correction to trace metal analysis of geochemical samples by atomic-absorption spectrophotometry. Econ. Geol. 65:588-589.

GAMULIN, T.
1956. Migrations of the Adriatic sardine in relation to zooplankton. *In* Pap. Presented Int. Tech. Conf. Conserv. Living Resour. Sea, Rome, 18 Apr.-10 May 1955, p. 338-340. U.N., N.Y.

GENERAL FISHERIES COUNCIL FOR THE MEDITERRANEAN.
1972. The state of marine pollution in the Mediterranean and legislative controls. FAO (Food Agric. Organ. U.N.), Stud. Rev. Gen. Fish. Counc. Mediterr. 51, 68 p.

GOLDBERG, E. D.
1972. Baseline studies of pollutants in the marine environment and research recommendations. IDOE (Int. Decade Ocean Explor.) baseline conference, May 24-26, 1972, N.Y., 54 p.

KECKES, S., AND J. K. MIETTINEN.
1972. Mercury as a marine pollutant. *In* M. Ruivo (editor), Marine pollution and sea life, p. 276-289. Fishing News (Books) Ltd., Surrey, Engl.

KNAUER, G. A.
1972. Trace metal relationships in a marine pelagic food chain. Ph.D. Thesis, Stanford Univ., 146 p.

KNAUER, G. A., AND J. H. MARTIN.
1972. Mercury in a marine pelagic food chain. Limnol. Oceanogr. 17:868-876.

KRAJNOVIĆ, M., AND D. DEKARIS.
1968. Hydrographic and biotical conditions in North Adriatic. VII. Racial analysis of the sardine (*Clupea pilchardus* Walb.) based on studies of erythrocyte antigens. Rapp. P.-V. Reun. Comm. Int. Explor. Mer Méditerr. 19:277.

LARRAÑETA, M. G.
1960. Synopsis of biological data on *Sardina pilchardus* of the Mediterranean and adjacent seas. *In* H. Rosa, Jr. and G. Murphy (editors), Proc. World Sci. Meet. Biol. Sardines Relat. Species, p. 137-173. FAO (Food Agric. Organ. U.N.), Rome.

LEONG, R. J. H., AND C. P. O'CONNELL.
1969. A laboratory study of particulate and filter feeding of the northern anchovy (*Engraulis mordax*). J. Fish. Res. Board Can. 26:557-577.

MAJOR, R. L.
1970. The Yugoslav fishery in the Adriatic Sea. Commer. Fish. Rev. 32(2):37-43.

MAJORI, L.
1968. Research experiments on the pollution of the waters of the upper Adriatic. *In* Int. Conf. Oil Pollut. Sea, 7-9 Oct. 1968, Rep. Proc., p. 349-381. Rome.

MAJORI, L., M. L. MORELLI, L. DIANA, AND G. RAUSA.
1967. L'inquinamento delle acque del mare nell'Alto Adriatico. I: Ricerche microbiologiche. Arch. Oceanogr. Limnol. 15(Suppl.):115-123.

MAJORI, L., M. L. MORELLI, G. RAUSA, AND L. DIANA.
1968. Recherches sur la pollution des eaux de mer dans la Golfe de Trieste. Rev. Int. Océanogr. Méd. 9:83-98.

MAJORI, L., G. RAUSA, M. L. MORELLI, AND L. DIANA.
1967. L'inquinamento delle acque del mare nell'Alto Adriatico. II: Ricerche chimiche. Arch. Oceanogr. Limnol. 15(Suppl.):125-134.

PANELLA, S.
1968a. Pollution of the ocean waters of Italy: causes, effects on the biological environment, control and prevention. Boll. Pesca Piscic. Idrobiol. 23(1):55-87.
1968b. Preliminary report on the pollution of Italian coastal waters in relation to the biological environment and fisheries. *In* Int. Conf. Oil Pollut. Sea 7-9 Oct. 1968, Rep. Proc., p. 50-67. Rome.
1970. Marine pollution in Italy. Boll. Pesca Piscic. Idrobiol. 25(1):193-217.

PICCINETTI, C.
1970. Considerazioni sugli spostamenti delle alici (*Engraulis encrasicholus L.*) nell'alto e medio Adriatico. Boll. Pesca Piscic. Idrobiol. 25(1):145-157.

RIVERS, J. B., J. E. PEARSON, AND C. D. SHULTZ.
1972. Total and organic mercury in marine fish. Bull. Environ. Contam. Toxicol. 8:257-266.

RUIVO, M. (editor).
1972. Marine pollution and sea life. Fishing New (Books) Ltd., Surrey, Engl., 624 p.

SELLI, R., M. FRIGNANI, C. M. ROSSI, AND R. VIVIANI.
1972. The mercury content in the sediments of the Adriatic and Tyrrhenian. *In* Journées Étud. Pollut., p. 39-40. Comm. Int. Explor. Sea. Méditerr., Athens.

STAINTON, M. P.
1971. Syringe procedure for transfer of nanogram quantities of mercury vapor for flameless atomic absorption spectrophotometry. Anal. Chem. 43:625-627.

ŠTIRN, J.
1965. Marine pollution in the Gulf of Trieste. Varstvo Narave 3:157-184.
1969. Pelagial Severnega Jadrana. Diss. Acad. Sci. Art. Slov., Vol. 12.
1970. Further contributions to the study of the bioproductivity in polluted marine ecosystems. Rev. Int. Océanogr. Méd. 18-19:21-27.

UI, J.
1971. Mercury pollution of sea and fresh water; Its accumulation into water biomass. Rev. Int. Océanogr. Méd. 22-23:79-128.

UI, J., AND S. KITAMURA.
1971. Mercury in the Adriatic. Mar. Pollut. Bull. 2(4): 56-58.

UTHE, J. F., F. A. J. ARMSTRONG, AND M. P. STAINTON.
1970. Mercury determination in fish samples by wet digestion and flameless atomic absorption spectrophotometry. J. Fish. Res. Board Can. 27:805-811.

ZAVODNIK, D.
1968. Contribution à la connaissance des migrations des Clupéidés dans l'Adriatique nord. Rapp. P.-V. Comm. Int. Explor. Mer Méditerr. 19:323-325.