

# The occurrence of selected chemical pollutants (Simazine, Lindane and Permethrin) in river fish

Progress report of first year's work July 1992

M. Ladle PhD I.S. Farr BSc



# INSTITUTE OF FRESHWATER ECOLOGY River Laboratory, East Stoke, Wareham, Dorset BH20 6BB

Tel: 0929 462314 Fax: 0929 462180

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M. Ladle & I.S. Farr

Project leader:

M. Ladle

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# **OBJECTIVES**

The ultimate objectives are:

- 1. To review the known occurrence of Simazine, Lindane and Permethrin in river fish.
- 2. To analyse fish species and/or age groups selected on the basis of spatial and temporal occurrence in order to determine the concentrations and variability of Simazine, Lindane and Permethrin in river fish.
- 3. To assess the significance of river fish in the environmental cycling of pesticides and to make recommendations on the role of fish in setting water quality standards.

#### **PROGRESS 1991-92**

Work on the project commenced in July 1991. Considerable delays were encountered in the early stages of the work due to difficulties in obtaining information. The following is a brief account of progress up to the present time.

#### Literature search

The Procite data base now contains references associated with this and related topics. In addition the literature has been examined with regard to methods currently in use for pesticide extraction, although, as mentioned in the previous report of progress, it is clear that most references in the field are strictly associated with toxicity tests. Some highly relevant data has recently been found, notably in a paper by Henderson, Inglis and Johnson (1972). In view of the nature of this North American study it was felt that it might be profitable to reanalyse the data in terms of:

- a. fish species which are known to occur in British rivers;
- b. the known feeding habits of all the species listed;
- c. the relationships between fish weights/lipid contents and pesticide levels.

Table 1 presents a summary of the available data. Appropriate analytical techniques will be applied and the literature search will now be directed toward unearthing similar information (if any exists).

## Studies by MAFF

A visit to discuss sampling and analysis procedures with Dr Colin Allchin of the MAFF fisheries laboratory at Burnham-on-Crouch was very valuable. It was quickly made clear to us that even in the marine field, knowledge of our area of research is rather limited, and thus that we will be effectively "breaking new ground". Comments as to the use of Atrazine and

Simazine on maize crops, Simazine for the maintenance of forestry fire breaks, and the extensive and regular detection of Lindane at many wet and dry raingauge sites and the occurrence of DDE in many samples taken from marine mammals provided useful background.

Clean-up procedures used by the MAFF scientists are akin to those which are employed in our analyses but the Soxhlet extraction used at Burnham-on-Crouch would be inappropriate for detection of Simazine.

# Information from the Health and Safety Executive

Information was obtained from the Health and Safety Executive on the uses of the various target pesticides. The following is a summary:

Simazine is a germination inhibitor used to prevent the growth of weeds. It is widely used by British Rail along tracks, by local authorities for clearing pavements, by fencing contractors prior to reseeding, in quarries, Anglian and Cambridge Water Companies who may have been monitoring broads and dykes for pesticides, The National Trust (Contact Ted Crawford Safety Coordinator of the National Trust) and by local authorities for clearing ground prior to the laying of tarmacadam. A ban on its future use is under consideration by MAFF Scientific and Medical Committee.

Permethrin (also Pyrethroids) is an insecticide used as a fumigant in glass houses and for industrial control of flies. Commercial names include Ambush (ICI). It is used as an insect powder for pets and other animals. The majority of cereal and rape growers will use one or other of the pyrethroids if they have an aphid problem. In horticulture it is used to control caterpillars, cabbage stem flea beetle and seed weevils.

Lindane is a wood preservative (woodworm killer) and may be used near rivers.

It was suggested that the ADAS agronomists could be asked whether they are being used in the proposed sampling areas this year (Mr Price, Blandford). "Crop Link" is an organisation with crop walkers who are "non-competent" agronomists used to identify weeds and pests affecting crops and to advise farmers on what action should be taken. B(B)W chemicals of Downton, Salisbury perform a similar function.

## Red List substances

Details of the occurrence of "Red List" substances were supplied by DoE. But the few instances of freshwater fish contamination proved to be of little value in planning the current research programme.

# Selection of sampling sites

Two possible sampling sites have now been selected on the basis of reported target pesticide inputs.

The river Stour, downstream of Kidderminster, in the Severn-Trent NRA region has already been studies in the course of IFE research. It is known that target pesticide pollution occurs in this area. Permissions for sampling have now been obtained with the assistance of Drs North and Hickley of the Severn-Trent regional NRA. As soon as the riparian owners grant access for sampling, an electric fishing team will be deployed to obtain samples of the available range of fish species.

Within the Thames NRA region there have been recent reports of pesticide pollution in the river Ray, Wiltshire. Again permissions to sample are being sought with the cooperation of the fisheries officer Vaughan Lewis and it is anticipated that the first samples will be obtained in the near future from a reach downstream of Swindon sewage treatment plant.

# Preliminary fish samples analysed

In the absence of concrete information on the incidence and location of contaminated fish, samples were obtained as outlined below and used to test analytical procedures and recovery efficiencies.

### FISH TISSUE ANALYSIS

#### Introduction

Literature surveys indicate that several methods are currently in use for the extraction, extract clean-up and analysis of pesticide residues in environmental samples. We report here tests of the recovery of three pesticides from fish tissue by two modified methods.

Both methods employ solvent extraction from the sample followed by a solid phase clean-up of the extract prior to analysis by GC with electron capture detection (ECD).

The compounds targeted in the present study represent three classes of pesticide; the triazine herbicide Simazine, the organochlorine insecticide BHC (lindane) and cis-permethrin, a pyrethroid insecticide.

The protocol employed at the Institute of Terrestrial Ecology, Monks Wood Experimental Station, was used for preliminary tests of the recovery of spike additions of the pesticides (Method 'A'). An extension of the method to allow measurement of Simazine was also tested.

The second method ('B') incorporates this extension as part of the clean-up procedure. The extract-clean-up in this method is a slight modification of that described by House *et al.* (1990), and the IFE report RL/T04053h1/2. This was developed for analysis of organochlorine, pyrethroid and triazine pesticide residues in extracts of aquatic sediments.

Method 'A' was developed for analysis of organochlorine insecticides and polychlorinated biphenyls in a wide range of environmental samples including animal tissues. Small quantities of tissue (c. 2 g wet weight) are required in the published method, an important consideration when limited populations are to be sampled.

Reasonable recoveries (30-90%) have been reported for organochlorines and other compounds with high octanol/water partition coefficients ( $K_{ow}$ ) using method 'A'. The unmodified method is unlikely to be suitable for analysis of the less lipophilic triazines since low polarity solvents are employed during extract clean-up.

#### Materials

Dace were obtained from the River Avon, Hampshire and Salmon and Thin Lipped Mullet from the River Frome, Dorset.

Fish were dissected and muscle, liver and other lipid-containing tissue stored separately at <-20°C prior to analysis.

Fish liver was initially selected as a suitable tissue to examine for the presence of accumulated pesticide residues.

Sand was acid washed (5N HCL) then heat treated at 700°C overnight. Initial tests used 0.5 mm sieved sand. 40-100 mesh sand (BDH) was used in method 'B'.

Anhydrous Na<sub>2</sub>SO<sub>4</sub> (Analar BDH) was dried at 160°C overnight.

Pesticide-analysis grade solvents were used throughout.

Glassware was cleaned in chromic acid, washed in distilled water and rinsed with three changes of acetone then hexane.

It was intended to test recovery of gamma BHC (lindane); however our initial tests with method 'A' used the closely related alpha isomer.

### Extraction Method 'A'

Samples of salmon liver (2.04 g) were weighed into 100 ml beakers and spiked with individual pesticides in acetone solution as follows:

| simazine       | 2.09 µg  | (10 pl | 209 mg/1 solution) |
|----------------|----------|--------|--------------------|
| a BHC          | 2.525 µg | (5 µl  | 505 mg/1 solution) |
| cis-permethrin | 2.505 µg | (5 µl  | 501 mg/1 solution) |

The samples were ground with sand (10 g). The resultant slurry was dried by adding anhydrous Na<sub>2</sub>SO<sub>4</sub> (>20 g) and stirring.

Extraction of the dry powder followed a modification to the published procedure already employed at Monks Wood, namely addition of alternate volumes of Hexane and Acetone (20 ml then 5 ml aliquots) decanting the previous extract before addition of the next aliquot until a volume of 50 ml had been collected.

Extracts are allowed to settle overnight then 25 ml subsamples taken and evaporated in a weighed vial with a stream of dry N<sub>2</sub>. Lipid content of the sample is calculated from the final weights of the vials.

### Extraction Method 'B'

Thin Lipped Mullet tissues were used to assess recovery efficiency of Method 'B'.

Weighed samples of liver and subcutaneous fatty tissue were ground with sand in the ratio of 2 g tissue to 10 g sand. Selected pairs of samples were spiked to either 100 or 200 µg/Kg (wet wt) with each pesticide.

All samples were then dried by stirring with Na<sub>2</sub>SO<sub>4</sub> as for method 'A' then left to complete drying at 5°C overnight.

1:1 acetone:hexane solvent was used for extraction as in the published method 'A'. Small volumes of the solvent were stirred with the dried sample, allowed to settle then decanted into a 100 ml volumetric flask. The procedure is repeated with 10 ml aliquots until 100 ml has been collected.

The cloudy extract is allowed to settle overnight at 5°C. The extract is then filtered through a precleaned No.4 porosity sintered glass filter leaving the sediment in the volumetric flask until the bulk of the extract has been filtered. The flask and filter holding the sediment are rinsed with fresh solvent and rinsings are filtered. 100% recovery of extract is assumed.

The filtrate is transferred quantitatively to a pre-weighed RB flask and evaporated to dryness on a rotary evaporator. The residue weight is determined and then taken up in 5% acetone in hexane (HA5) prior to the first stage clean-up.

## Extract clean-up Method 'A'

Evaporated extracts are dissolved in hexane (5 ml) and a 1 ml sample transferred to a column of preconditioned aluminium oxide (0.8 g, Neutral Brockmann grade 1) in a pasteur pipette. The aluminium oxide was conditioned by heating to 800°C (4 hrs), cooling, then deactivating with 5% water (w/w).

Lipophilic compounds are eluted with hexane (5x 1 ml aliquots). The eluate is analysed directly.

Simazine is not readily soluble in hexane and this procedure is unlikely to give acceptable recovery of the material. Previous work has shown that sediment extracts containing simazine can be successfully cleaned in 5%-10% acetone in hexane solution using activated magnesium silicate (Florisil) solid phase (House *et al.* 1990 and IFE report RL/T04053h1/2).

The remaining 4 ml of the dried extract in hexane was evaporated to dryness under  $N_2$  and taken up in 10% acetone in hexane (5 ml). A 2 ml aliquot of this solution was applied to an activated florisil column following the procedure for the second stage of the clean-up protocol described in the method of House *et al.* (1990). The column was eluted with 10% acetone in hexane (HA10 40 ml) and the eluate evaporated to low volume before making up to 5 ml with 10% acetone in hexane and analysing.

# Extract clean-up Method 'B'

In the first stage of the method 'B' clean-up, the extracts in HA5 are transferred to a florisil column (Bondelut 3 ml) preconditioned with HA5 (20 ml). The RB flask was rinsed with successive 5 ml aliquots of HA5 to a total volume of 60 ml. Rinsings were used to elute pesticides from the column.

The collected eluates are returned to the RB flask and evaporated to dryness on the rotary evaporator.

The residue was extracted with 5 ml aliquots of hexane to a total volume of 20 ml. Hexane extracts were transferred to an activated florisil column (as in method 'A') and the hexane eluate collected (wash eluate). Pesticides residues in the RB flask were transferred to the column with successive 5 ml washes with HA10. Three 20 ml HA10 eluates were collected for separate analysis (eluates 1, 2 and 3 EL) to determine the elution pattern of the added pesticides.

10 ml subsamples of the eluates were taken and evaporated to dryness under  $N_2$ , spiked with phosalone as internal standard and made up to 1 ml with HA10 for analysis.

## Analyses

Pesticide residues were analysed by gas liquid chromatography (GLC) on a Perkin Elmer 8700 gas chromatograph fitted with a split/splitless injector, 25 m DB5 capillary column (5% phenylmethyl silicone stationary phase) and an electron capture detector (ECD).

Chromatographic conditions were as reported in House *et al.* 1990. Compounds were identified by relative retention time (RRT) with reference to an internal standard, aldrin. External standards were used to calibrate the method for quantification.

Simazine analysis using the above conditions can be inconsistent. Analyses for simazine reported here used the same instrument with a programmable temperature vaporizer (PTV) injector, 30 m DB1301 column and nitrogen phosphorus detector (NPD) which has a greater sensitivity than ECD for this compound.

Chromatographic conditions were as follows:

# Oven temperatures:

Initial temperature 140°C iso time 1 minute

First ramp 20°C per minute Isothermal 240°C for 7 minutes

## Injector conditions:

Initial temperature 100°C for 1 minute

in split mode

Vaporization temperature 280°C for 5 minutes

in splitless mode

Final temperature 150°C for 1 minute

# Gases:

Makeup N<sub>2</sub>
Carrier He

Septum purge =  $5 \text{ ml min}^{-1}$ Flow rate =  $50 \text{ ml min}^{-1}$ 

External standards were used for quantification and identification on the basis of retention time (+ 0.02 minutes).

A mass selective detector (HP 4971A attached to a HP 5890 series II GC) run in selective ion mode was used for further confirmation of the presence of pesticides. Two characteristic ions were selected to monitor each pesticide.

#### Results Method 'A'

No simazine, cis-permethrin or BHC were detected in extracts of unspiked salmon liver.

% Recovery of pesticide spikes

| Compound                    | Spiked liver sample 1 | Spiked liver sample 2 | Spiked<br>- blank |
|-----------------------------|-----------------------|-----------------------|-------------------|
| ВНС                         | 107.3                 | 105.7                 | 65                |
| cis-permethrin              | 32.1                  | no result             | 34.5              |
| simazine:                   |                       |                       |                   |
| original clean-up<br>method | <del>-</del>          | <del>-</del>          | <del>-</del>      |
| modified clean-up<br>method | 57,6, 51.3            | 52.1                  | 57.2, 66.8        |

Recovery of BHC from liver appears quantitative although there is some uncertainty about the spiked blank.

Cis-permethrin recovery is low using this protocol. The modified clean-up procedure used for simazine analysis will be examined to see if losses are occurring at the clean-up stage.

No simazine could be detected in the extracts prepared using the published clean-up method. The modified clean-up yielded acceptable and reasonably consistent recoveries for all spiked sample extracts.

Traces of several organochlorine pesticides were detected in the spiked and unspiked liver extracts and solvent blank extracts.

Only Dieldrin was found in all samples. Liver extracts indicated levels of between 1 and 1.25 µg/kg of liver. However blank extracts contained higher levels and trace contamination cannot be ruled out.

Heptachlor was detected in the unspiked liver (1.25  $\mu$ g kg), one sample of spiked liver (<0.2  $\mu$ g kg); also in the blank extract (0.125  $\mu$ g, a high level which may indicate misidentification).

Traces of lindane were found in one spiked liver extract (1.5 µg kg) and the solvent blank (equivalent to 1 µg kg).

Traces of DDT, DDE, endrin and trans-permethrin were also detected but not consistently in liver extracts or were present at similar levels in the blank.

## Results Method 'B'

Patterns of elution of the three pesticides applied as spikes to Mullet fatty tissue are shown in Fig. 1. The first two 20 ml HA10 eluates contain essentially all of the eluted pesticide residues.

Spike recoveries corrected for traces measured in the unspiked samples are given in the following table:

% Recovery of 200 µg kg<sup>-1</sup> spike in eluates

| Compound       | wash eluate | 1EL   | 2EL  | 3EL  | Combined |
|----------------|-------------|-------|------|------|----------|
| Simazine       | 0           | 66.4  | 19.3 | 0    | 85.4     |
| Lindane        | 0           | 62.6  | 0.95 | 0.75 | 64.3     |
| Cis-permethrin | 0           | 55.35 | 3.38 | 1.67 | 60.4     |

Relatively high levels of pesticide spikes were used in the tests reported here; 1024 to 1228 µg kg<sup>-1</sup> for method 'A' and 200 µg kg<sup>-1</sup> for method 'B'. Injected concentrations however approached the limits of reliable quantitation for cis-permethrin and BHC in method 'A'. Sensitivity was considerably greater for method 'B' where a greater proportion of the extract was used in the clean-up stage. Recovery of lindane was lower in method 'B' than 'A' though within acceptable limits.

#### PRESENT STATUS OF STUDY

Although there are considerable difficulties due to the absence of published information on many aspects of this work, we are now in a position to commence taking and analysing samples from rivers and have located sites which should provide suitable material for analysis. It is anticipated that sampling will commence in late August 1993.

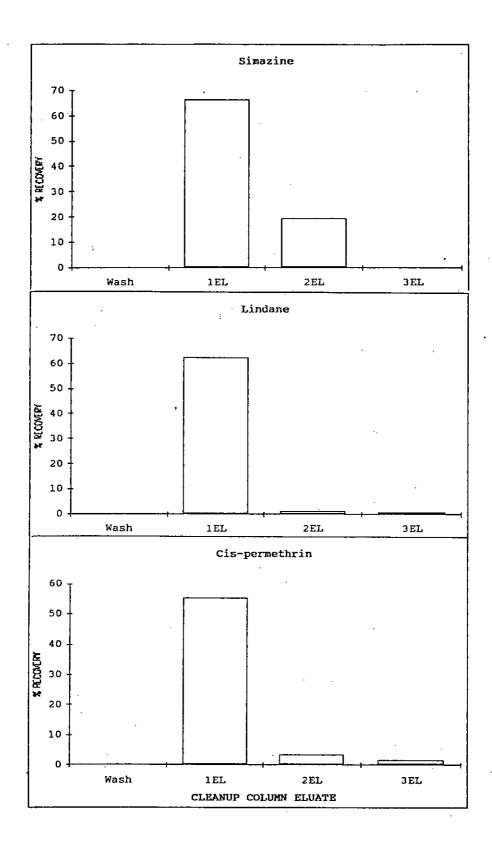


Figure 1. Elution pattern of pesticides from final clean-up column - Method B. Pesticide spikes (200 µg kg<sup>01</sup>) extracted from Mullet subcutaneous fatty tissue.

Table 1. Extracted details of fish analyses after Henderson, Inglis and Johnson (1971)

| Species                        | Length  | Weight  | Lipid%       | HCH(ppm)                | DDE(ppm)     | <u> </u>                                       |             |
|--------------------------------|---------|---------|--------------|-------------------------|--------------|--|-------------|
| Arctic grayling                | 11.7    | 0.5     |              |                         | 0.25         |  |             |
| Arctic grayling                | 11.7    | 0.5     | ·            |                         | 0.195        | [  |             |
| Bigmouth buffalo               | 16.7    | 2.7     | 6.22         |                         | 0.15         |  |             |
| Bigmouth buffalo               | 15.7    | 2       | 10.9         |                         | 0.62         |  |             |
| Bigmouth buffalo               | 16.2    | 2.35    | 8.56         | 0.05                    | 0.385        |  |             |
| Black bullhead                 | 5.4     | 0.2     | 1.51         | 0.38                    | 0.03         |  |             |
| Black bullhead                 | 9.8     |         |              | 0.01                    | 0.03         | <u> </u>                                       | ·           |
| Black bullhead                 | 7.6     | 0.35    |              | 0.195                   | 0.035        | ¦  |             |
|                                | 10.4    | 0.33    | 6.07         | 0.193                   | 0.033        | <del> </del>                                   |             |
| Black crappie<br>Black crappie | 7.4     | 0.3     | 8.07<br>88.1 |                         | U.62<br>U.94 | ļ  |             |
| Black crappie                  | 7       | 0.3     | 3.9          | 0.03                    | 0.4          |  |             |
| Black crappie                  | 8.26667 | 0.4     | 3.95         |                         | 0.65333      | l  | <del></del> |
| Bloater                        | 12      | 0.8     | 26.5         |                         | 3.52         | i  |             |
| Bloater                        | 11.2    | 0.4     | 12.1         | 0.03                    | 1.07         | j  |             |
| Bloater                        | 11.6    | 0.6     | 19.3         | 0.055                   | 2.295        | <u> </u>                                       |             |
| Blue catfish                   | 13.5    | 0.7     |              |                         | 1.87         |  |             |
| Blue catfish                   | 13.5    | 0.7     | 1.64         | 0.14                    | 1.87         | ii   |             |
| Bluegill                       | 6.8     | 0.2     | 0.7          |                         | 0.36         | <del> </del>                                   |             |
|                                | 7.3     | 0.2     | 4.39         |                         |              | ļ  |             |
| Bluegill                       |         |         |              | 0.2                     | 0.22         |  |             |
| Bluegill                       | 7.6     | 0.4     | ·            |                         | 0,81         |  |             |
| Bluegill                       | 6.2     | 0.2     | 1.65         | 0.03                    | 0.15         |  |             |
| Bluegill                       | 6.2     | 0.1     | 1.21         | 0.02                    | 0.21         |  |             |
| Bluegill                       | 7.1     | 0.4     |              | 0.01                    | 0.04         | <u> </u>                                       |             |
| Bluegill                       | 6.86667 | 0.26667 | 2.65167      | 0.065                   | 0.29833      | l<br>i   |             |
| Bridgelip sucker               | 14      | 1.5     | 6.96         | 0.02                    | 0.35         | !  |             |
| Bridgelip sucker               | 14      | 1.5     | 6.96         | 0.02                    | 0.35         |  |             |
| Brown bullhead                 | 11.7    | 0.9     | 8.54         | 0.18                    | 1.65         | ļ ———— Ì                                       |             |
| Brown bullhead                 | 9.6     | 0.4     | 2.34         | 0.01                    | 0.26         |  |             |
| Brown bullhead                 | 10.8    | 0.6     | 2.09         | 0.05                    | 0.23         | 1  |             |
| Brown bullhead                 | 11.9    | 0.8     | 5.9          | 4.37                    | 0.17         |  |             |
| Brown bullhead                 | 10.2    | 0.7     | 4.47         | 0.02                    | 80.0         |  |             |
| Brown bullhead                 | 9.5     | 0.5     | 3.72         | 0.02                    | 0.86         |  |             |
| Brown bullhead                 | 10.6167 | 0.65    | 4.51         |                         | 0.54167      |  |             |
| Carp                           | 20      | 3.5     | 2.38         | 0.01                    | 0.24         |  |             |
| Carp                           | 15.1    | 1.9     |              | 0.01                    | 0.24         | <del> </del>                                   |             |
| Carp                           | 15.8    | 1.8     | 3.15         | <del></del>             | 0.3          | ·  |             |
| Carp                           | 20      | 4.6     | 6.15         | ·                       | 2.93         |  | ···         |
| } <del></del>                  | 13.4    | 1.7     |              | 0.08                    |              |  |             |
| Carp<br>Carp                   | 16.3    |         |              | 0.99                    | 0.06         |  |             |
|                                |         | 2.1     | 11.7         | 0.04                    | 0.3          |  |             |
| Carp                           | 8.6     | 0.4     |              | 0.31                    | 80.0         |  |             |
| Carp                           | 10.1    | 1.6     | 9.92         | 0.22                    | 0.39         |  | ·           |
| Carp                           | 11.8    | 0.8     | 2.59         | 0.03                    | 0.29         | <u> </u>                                       |             |
| Carp                           | 15.3    | 1.9     | 5.76         | 0.06                    | 1.74         |  |             |
| Carp                           | 13.9    | 1.4     | 3.61         | 0.01                    | 0.06         |  |             |
| Carp                           | 21      | 3.8     | 3.6          | 0.19                    | 1.2          |  |             |
| Carp                           | 14.5    | 1.5     | 1.59         | 0.01                    | 0.07         |  |             |
| Carp                           | 24      | 7.5     |              | 0.07                    | 0.75         |  |             |
| Carp                           | 13.6    | 1.2     | 5.46         | 0.02                    | 0.25         |  |             |
| Carp                           | 15.2    | 1.6     | 7.05         | 0.01                    | 0.03         |  |             |
| Carp                           | 11      | 0.9     | 2.5          | *                       | 0.04         |  |             |
| Carp                           | 16.9    | 2.5     | 5.3          | 0.01                    | 0.26         | <del></del>                                    |             |
| Carp                           | 14.6    | 1.5     | 4.98         | 0.01                    | 0.13         |  |             |
| Carp                           | 17      | 2.1     | 8.51         | 0.01                    | 0.1          |  |             |
| Carp                           | 12.9    | 0.9     | 4.14         | TOTAL TERMS OF LANGUAGE | 0.94         |  |             |
| Carp                           | 14.7    | 1.3     |              |                         | 0.86         | <del></del>                                    |             |
| Oai p                          | 14.7    | 1.3     | 1.99         |                         | 0.86         | <u>.                                      </u> |             |

| Carp              | 15.2591 | 2.11364 | 5.88545      | 0.12053 | 0.51818 |  |
|-------------------|---------|---------|--------------|---------|---------|--|
| Chain pickerel    | 13.7    | 0.5     | 1.3          | 0.07    | 0.06    |  |
| Chain pickerel    | 13.7    | 0.5     | 1.3          | 0.07    | 0.06    |  |
| Channel catfish   | 13.6    | 0.9     | 9.65         |         | 0.3     |  |
| Channel catfish   | 21.5    | 3.91    | 8.39         | 0.2     |         |  |
| Channel catfish   | 11      | 0.9     | 7.52         | 0.01    | 0.04    | ·                                      |
| Channel catfish   | 27      | 10      | 10.4         | 0.03    | 42.3    |  |
| Channel catfish   | 11.6    | 0.9     | 6.18         | 0.03    | 0.56    |  |
| Channel catfish   | 13      | 0.9     | 20,1         | 1.5     | 0.09    |  |
| Channel catfish   | 15.2    | 0.9     | 4.95         | 0.06    | 2.93    |  |
| Channel catfish   | 15.9    | 1.5     | 14.8         | 0.29    | 0.7     |  |
| Channel catrish   | 15.7    | 1.3     |              | 0.29    | 0.75    |  |
|                   | 14.5    | 0.8     | 8.74<br>5.83 |         |         |  |
| Channel catfish   |         |         |              | 0.01    | 0.49    |  |
| Channel catfish   | 13      | 0.8     | 8.88         | 0.01    | 0.06    |  |
| Channel catfish   | 9.2     | 0.2     | 4.2          | 0.01    | 0.61    |  |
| Channel catfish   | 16.1    | 1.2     | 5.65         | 0.01    | 0.78    |  |
| Channel catfish   | 15.1769 | 1.86154 | 8.86846      | 0.22769 | 3.89538 |  |
| Chiselmouth       | 10.2    | 0.5     | 2.98         | 0.03    | 0.14    |  |
| Chiselmouth       | 7.9     | 0.3     | 7.67         | 0.02    | 0.7     | <u> </u>                               |
| Chiselmouth       | 7.9     | 0.3     | 7.67         | 0.02    | 0.7     |  |
| Flannelmth sucker | 19.2    | 2.6     | 8.97         | 0.02    | 0.13    |  |
| Flannelmth sucke  | 19.2    | 2.6     | 8.97         | 0.02    | 0.13    |  |
| Flathead catfish  | 21      | 4.6     | 6.68         | 0.02    | 0.82    |  |
| Flathead catfish  | 21      | 4.6     | 6.68         | 0.02    | 0.82    |  |
| Freshwater drum   | 13.5    | 1.1     | 6.21         | 0.02    | 0.26    |  |
| Freshwater drum   | 13.5    | 1,1     | 6.21         | 0.02    | 0.26    |  |
| Gizzard shad      | 12      | 0.6     | 2.31         | 0.1     | 0.27    |  |
| Gizzard shad      | 11.4    | 0.6     | 4.4          | 0.06    | 1.54    |  |
| Gizzard shad      | 11.7    | 0.6     | 3.355        | 0.08    | 0.905   |  |
| Goldeye           | 12.5    | 0.8     | 13.8         | 0.05    | 0.23    |  |
| Goldeye           | 10.8    | 0.3     | 14           | 0.02    | 0.03    |  |
| Goldeye           | 12.9    | 0.5     | 12.5         | 0.08    | 0.29    |  |
| Goldeye           | 12.0667 | 0.53333 | 13,4333      | 0.05    | 0.18333 |  |
| Goldfish          | 11.7    | 1.5     | 12.1         | 0.51    | 1.24    |  |
| Goldfish          | 11.7    | 1.5     | 12.1         | 0.51    | 1.24    |  |
| Klamath sucker    | 13.5    | 1.2     | 3.99         |         | 0.02    |  |
| Klamath sucker    | 13.5    | 1.2     | 3.99         | #DIV/0! | 0.02    |  |
| Lake trout        | 22      | 3       | 12           | 0.02    | 0.98    |  |
| Lake trout        | 14.6    | 0.9     | 2.64         | 0.01    | 0.04    |  |
| Lake trout        | 18.3    | 1.95    | 7.32         | 0.015   | 0.51    |  |
| Lake whitefish    | 16.1    | 1.2     | 13.2         | 0.05    | 0.34    |  |
| Lake whitefish    | 16.1    | 1.2     | 13.2         | 0.05    | 0.34    |  |
| Largemouth bass   | 9.2     | 0.5     | 2.93         | 0.27    | 1.34    |  |
| Largemouth bass   | 10.5    | 0.5     | 0.44         | 0.01    | 0.17    |  |
| Largemouth bass   | 9.7     | 0.6     | 2.6          | 0.01    | 0.72    |  |
| Largemouth bass   | 13.4    | 1.3     | 5.49         | 0.2     | 1.63    |  |
| Largemouth bass   | 10.5    | 0.8     | 2.45         | 0.2     | 0.37    | ************************************** |
| Largemouth bass   | 17.3    | 3.2     | 9.26         | 0.01    | 0.12    | <del> </del>                           |
| Largemouth bass   | 13.4    | 1.6     | 2.88         |         | 0.12    |  |
| Largemouth bass   | 15.6    | 2.7     | 9.86         | 0,07    | 1.26    |  |
| Largemouth bass   | 14      | 1.5     | 4.53         | 0.12    | 5.85    |  |
| Largemouth bass   | 11.3    | 0.7     | 7.11         | 0.12    | 1.69    |  |
| Largemouth bass   | 11.8    | 0.7     | 1.4          |         |         |  |
|                   | 12      |         |              | 0.02    | 0.59    |  |
| Largemouth bass   |         | 1       | 1.03         |         | 0.1     |  |
| Largemouth bass   | 15      | 2.5     | 3.77         | 0.09    | 0.12    |  |
| Largemouth bass   | 10.3    | 0.5     | 1.66         | 0.01    | 0.12    | <u>!</u>                               |

| Largemouth bass                        | 12.3         | 1.1        | 4.77            | 0.03                                   | 0.19         |             |                 |
|--|--------------|------------|-----------------|--|--------------|-------------|-----------------|
|  | 10.8         | 0.6        |                 | !                                      | 0.13         |             |                 |
| Largemouth bass                        |              |            |                 |  |              |             |                 |
| Largemouth bass                        |              | 1.24375    | 3.87563<br>4.77 |  |              | ·           |                 |
| Largescale sucker                      | 15<br>15.2   | 1.4<br>1.6 | 4.77            | 0.01                                   | 0.33<br>0.14 | <br>        |                 |
| Largescale sucker<br>Largescale sucker | 13.2         | 0.9        | 4.75            | 0.01                                   | 0.14         |             |                 |
|  | 13.9         | 1.5        | 10.8            | 0.01                                   | 0.17         | ~           |                 |
| Largescale sucker                      | 16.5         |            | 5.25            |  | 0.13         | <u> </u>    |                 |
| Largescale sucker                      | <del> </del> | 21         |                 | 0.01                                   |              | <br>        |                 |
| Largescale sucke                       |              | 1.48       | 6.034           | 0.0375                                 | 0.29         |             | ·               |
| Longnose sucker                        | 15           | 1.2        | 3.02            | 0.03                                   | 0.54         | !           |                 |
| Longnose sucker                        | 15.5         | 1.5        | 1.53            | 0.01                                   | 0.01         |             |                 |
| Longnose sucker                        |              | 1.35       | 2.275           | 0.02                                   | 0.275        |             | ····            |
| Northern Squawfis                      | 13.1         |            | 4.91            | 0.01                                   | 1.87         |             |                 |
| Northern Squawfi                       |              | 1          | 4.91            | 0.01                                   | 1.87         |             |                 |
| Pumpkinseed                            | 6.2          | 0.2        | 3.03            | 0.09                                   | 0.23         |             | - · <del></del> |
| Pumpkinseed                            | 6.2          | 0.2        | 3.03            | 0.09                                   | 0.23         |             | <del></del>     |
| Rainbow trout                          | 13.2         | 1!         | 6.42            | 0.01                                   | 0.5          | ·           |                 |
| Rainbow trout                          | 11.6         | 0.8        | 6.09            | 0.01                                   | 80.0         |             |                 |
| Rainbow trout                          | 13.2         | 0.9        | 5.48            | 0.01                                   | 0.08         |             |                 |
| Rainbow trout                          | 12.6667      | 0.9        | 5.99667         | 0.01                                   | 0.22         | L           |                 |
| Redbreast sunfish                      | 5.8          | 0.2        | 2.38            |  | 0.02         |             |                 |
| Redbreast sunfist                      | 5.8          | 0.2        |                 | #DIV/0!                                | 0.02         | <u> </u>    |                 |
| Redhorse sucker                        | 19           | 2.8        | 5.63            | 0.02                                   | 0.36         |             |                 |
| Redhorse sucker                        | 7.9          | 0.04       | 2.25            | 0.18                                   | 0.17         |             |                 |
| Redhorse sucker                        | 16.9         | 2          | 7.88            | 0.02                                   | 0.03         |             |                 |
| Redhorse sucker                        | 14.6         | 1.61333    | 5.25333         | 0.07333                                | 0.18667      |             |                 |
| Rock bass                              | 7.2          | 0.3        | 2.33            | 0.01                                   | 0.08         |             |                 |
| Rock bass                              | 8.6          | 0.6        | 5.77            | 0.14                                   | 0.6          |             |                 |
| Rock bass                              | 7.9          | 0.45       | 4.05            | 0.075                                  | 0.34         |             |                 |
| Round Whitefish                        | 10           | 0.2        | 3.72            | 0.04                                   | 0.27         |             |                 |
| Round Whitefish                        | 10           | 0.2        | 3.72            | 0.04                                   | 0.27         |             |                 |
| Sauger                                 | 13.7         | 0.9        | 4.93            | 0.01                                   | 0.38         | i           |                 |
| Sauger                                 | 13.7         | 0.9        | 4.93            | 0.01                                   | 0.38         |             |                 |
| Smallmouth bass                        | 15.5         | 1.3        | 3.06            |  | 0.94         |             |                 |
| Smallmouth bass                        | 10           | 0.9        | 4.58            | 0.01                                   | 0.94         |             |                 |
| Smallmouth bass                        | 12.75        | 1.1        | 3.82            | 0.01                                   | 0.94         |             |                 |
| Smallmouth buffalo                     | 16.3         | 2.5        | 8.54            | 0.08                                   | 0.46         | <del></del> |                 |
| Smallmouth buffa                       | 16.3         | 2.5        | 8.54            | 0.08                                   | 0.46         |             |                 |
| Spotted sucker                         | 12.6         | 1.2        | 3.49            | 0.00                                   | 0.29         |             |                 |
| Spotted sucker                         | 17.4         | 2.3        | 4.94            |  | 0.45         | r           |                 |
| Spotted sucker                         | 15           | 1.75       | 4.215           | 0.065                                  | 0.43         |             |                 |
| Striped mullet                         | 16           | 1.73       | 8.07            | 0.083                                  | 4.55         |             |                 |
| Striped mullet                         | 14.3         | 1.1        | 7.64            | 1.14                                   | 0.08         |             |                 |
| Striped mullet                         | 15.15        | 1.45       | 7.855           | 0.71                                   | 2.315        |             |                 |
| Walleye                                | 17.2         | 1.45       | 4.7             |  | 0.42         |             |                 |
| Walleye                                | 17.6         | 1.4        | 5.03            | 0.01                                   | 0.42         | ·····       |                 |
| Walleye                                | 17.4         | 1.5        | 4.865           | 0.01                                   | 0.05         |             |                 |
| White bass                             | 10.2         | 0.5        | 2.87            | 0.01                                   | 0.233        |             |                 |
| White bass                             | 10.2         | 0.5        | 2.87            | 0.01                                   |              |             |                 |
| White catfish                          | 12           |            |                 |  | 0.13         |             |                 |
|  |              | 0.9        | 5.94            | 0.23                                   | 0.38         |             |                 |
| white catfish                          | 14.1         | 1.3        | 5.56            | ······································ | 0.86         |             |                 |
| white catfish                          | 13.05        | 1.1        | 5.75            | 0.23                                   | 0.62         | <u> </u>    |                 |
| White crappie                          | 7.6          | 0.2        | 2.08            | 2.19                                   | 0.03         |             |                 |
| White crappie                          | 8.9          | 0.4        | 3,55            | 0.07                                   | 0.23         |             |                 |
| White crappie                          | 8.7          | 0.2        |                 | *                                      | R            |             |                 |
| White crappie                          | 6.9          | 0.2        | 1.76            | 0.07                                   | 0.22         |             | -               |

| White crappie | 8.025    | 0.25              | 2.46333 | 0.77667 | 0.16    |          |
|---------------|----------|-------------------|---------|---------|---------|----------|
| White perch   | 10       | 0.6               | 5.13    | 0.25    | 0.64    |          |
| White perch   | 9.4      | 0.5               | 6.84    | 0.18    | 10.9    |          |
| White perch   | 9.5      | 0.5               | 9,68    | 0.26    | 1.95    |          |
| White perch   | 9.63333  | 0.53333           | 7.21667 | 0.23    | 4.49667 |          |
| white sucker  | 15       | 1.5               | 4.22    | 0.22    | 0.05    |          |
| White sucker  | 14.7     | 1.4               | 1.85    | 0.2     | 4.82    |          |
| White sucker  | 12.7     | 0.8               | 5.27    | 0.01    | 0,1     |          |
| White sucker  | 15.1     | 1.5               | 2.95    | 0.2     | 0.22    |          |
| White sucker  | 14.8     | 1.5               | 5.07    | 0.01    | 0.43    |          |
| White sucker  | 12.1     | 0.7               | 4.36    | 0.2     | 2.3     |          |
| White sucker  | 16.4     | 2.1               | 4.23    | 0.01    | 0.08    |          |
| White sucker  | 14.4     | 1.35714           | 3.99286 | 0.12143 | 1.14286 |          |
| Yellow perch  | 7.7      | 0.2               | 4.35    | 0.25    | - 0.1   |          |
| Yellow perch  | 8.8      | 0.4               | 5.96    | 0.2     | 0.49    | <u> </u> |
| Yellow perch  | 9.1      | 0.4               | 3.08    | 0.02    | 0.31    |          |
| Yellow perch  | 10.4     | 0.6               | 7.21    | 0.31    | 0.75    | 1        |
| Yellow perch  | 9.4      | 0.4               | 4.64    | 0.01    | 0.35    |          |
| Yellow perch  | 9.9      | 0.5               | .3.58   | 0.02    | 0.48    |          |
| Yellow perch  | 10.3     | 0.6               | 6.69    | 0.05    | 2.41    |          |
| Yellow perch  | 8.9      | 0.4               | 3.69    | 0.01    | 0.03    |          |
| Yellow perch  | 9.3125   | 0.4375            | 4.9     | 0.10875 | 0.615   |          |
|               |          |                   |         |         |         |          |
|               |          |                   |         |         |         |          |
|               |          |                   |         |         |         |          |
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|               | <b> </b> |                   |         |         |         | <br>     |
|               | <u> </u> | <u>_</u> <u>_</u> |         |         |         | <br>1    |

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